

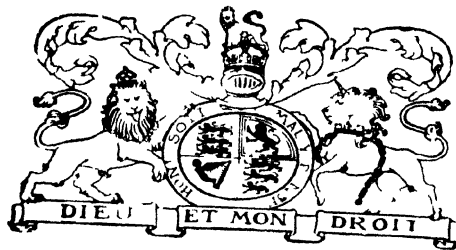


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RECORDS OF THE MALARIA SURVEY OF INDIA.

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MALARIA RESEARCH AND THE MALARIA COMMISSION OF THE LEAGUE OF NATIONS.

[5th January, 1934.]

WE have been asked by the Secretary to the Malaria Commission of the League of Nations to publish the following summary of the work done by the Commission and the programme of investigation recommended for the future.

Editor.

THIS year malariologists will have noticed the appearance of several important reports published by the Malaria Commission of the League of Nations, or under its auspices.

Organized ten years ago, the Malaria Commission began by studying the epidemiology of malaria as well as all the methods of malaria control employed in different countries in many of which the disease had assumed epidemic proportions as a result of the world war.

The first two reports (Malaria Commission, 1925; 1927) therefore deal with the epidemiology of malaria in the countries in which investigations had been carried out on its behalf and on the principles governing the prevention of malaria. The third general report (Malaria Commission, 1933) deals with the Therapeutics of Malaria and describes the present status of our knowledge of the treatment of the disease and the opinions of the Commission on methods of applying that treatment.

A Report on 'Housing and Malaria' by Christophers and Missiroli (1933) deals with a problem that was taken up by the Commission in 1928. Another subject, Malaria in the Deltas of large rivers, has been investigated for several years by members of the Commission. Reports have been published on the deltas of the Danube (Zotta, 1932), the Ebre (Pittaluga *et al.*, 1932), and the Rhine (Swellengrebel, 1933), followed by a memorandum on certain 'General Considerations' on the problem (Cantacuzéne *et al.*, 1933).

These different reports define the limits of our present knowledge on these three subjects, but as that knowledge is still far from complete, and malarial research still actively in progress, the Commission feels that lines of further investigation should be indicated in order to point out the problems most urgently calling for solution.

The full list of questions on which the Commission recommends that research should be undertaken is given below. It will be noted that all the problems are classified under the three subjects which have been investigated

for many years, *vis.*, (i) Treatment of Malaria, (ii) Housing and Malaria, and (iii) Malaria in Deltas.

The study of these problems is not the monopoly of the League Malaria Commission. The members of that Commission feel strongly that they should draw the attention of investigators all over the world to the importance of these problems. No malariologist denies that malaria is essentially a local problem: no country is alike as regards malaria. Therefore, the results of any investigation apply as a rule only to the country where it was made, and if conclusions of a more general value are to be reached, the observations and controlled experiences of different national investigations should first be pooled.

For example, immunity to malaria varies, of course, according to the greater or lesser opportunity of repeated infection, to the virulence of the strain, to the number of sporozoites inoculated, and so on. Malariatherapy offers a good opportunity for the study of this problem, but it is of the greatest interest to know if a strain of, let us say, *P. vivax* which has proved very virulent in England, has the same virulence in Roumania or in Italy; or if a Roumanian strain towards which patients have become immune in Roumania, as the result of repeated inoculation, protects against the English strain, and *vice versa*.

The international co-ordination of research into malaria has proved to be of the greatest value; to carry it out, however, would be difficult, if not impossible, without the existence of an international organization of some sixty members and corresponding experts like the Malaria Commission of the League

The following is the list of questions recommended for further investigation :—

(i) *Treatment—clinical and therapeutical research work.*

Determination in hyperendemic and endemic regions of the age groups most seriously affected by the disease and consequently requiring most attention in the matter of treatment. Investigations should cover in the first place the indigenous population and then be extended to immigrants.

Determination of the minimum dose of quinine sufficient for the treatment of the disease in hyperendemic and endemic areas where the natives have attained a certain degree of immunity

Ascertainment of the dosage (by age groups) of plasmoquine sufficient to prevent the gametocytes from infecting the *Anopheles* and the intervals (per week) at which it should be administered.

Determination of the extent, if any, to which the therapeutical action of quinine is increased by the administration of plasmoquine (combined medication).

A beginning should be made with laboratory research on cases of induced infection before proceeding to apply this method in the field.

Continuation of clinical tests with both types of 'totaquina' in accordance with the method laid down in the report.

Experiments in malaria control by means of medicaments alone without the application of anti-anopheline measures.

Blackwater fever; investigations of the relations between quinine and blackwater fever by means of experiments on animals (malaria in monkeys).

(ii) *Housing and Malaria.*

Biology and geographical distribution of the different varieties of *Anopheles maculipennis*.

Study of the following species of tropical *Anopheles* from the point of view of a possible differentiation of races :

- (a) *A. hyrcanus* and its several varieties (though widely disseminated, this strain is not as a rule very dangerous, though it may become so in certain countries, and especially Sumatra).
- (b) *A. bifurcatus* (unimportant in Europe, but dangerous in Palestine).
- (c) *A. ludlowi* var. *sundaicus* (dangerous everywhere, though there is a great difference between its breeding-places on the coast of Java and in the interior of Sumatra).
- (d) *A. gambiae* (dangerous everywhere, though its breeding-places differ in the Union of South Africa and in Tropical Africa).

Causes of the very great variations met with in the distribution of malaria in certain tropical regions—such as the phenomenon of immune areas in the immediate vicinity of hyperendemic areas—and in particular the possibility of a relation existing between this phenomenon and the deviation of the *Anopheles*.

Investigation of the factors which make certain rice-growing areas highly subject to malaria while others remain immune.

Study of the African *Anopheles* and the connection between the various species of *Anopheles* and malaria in Africa.

(iii) *Malaria in Deltas.*

Initiation or pursuit of research on the varieties of *A. maculipennis* found in European deltas in connection with malarial foci (Danube, Ebro, Rhine, Rhone, Po).

It is suggested that Indian, Siamese and Indo-Chinese malariologists might usefully conduct similar investigations with regard to the races of malaria-carrying *Anopheles* in the deltas of their respective countries.

Investigation of the influence of agriculture on the domesticity of *Anopheles* and on malarial endemicity.

Study of live stock in connection with local anophelism and the disease.

Historical study with special reference to malaria of variations in the topographical and demographical characteristics of deltas.

Investigation of the degree of susceptibility to malarial infection of inhabitants of deltaic areas.

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4 *Summary of Research Work by Malaria Commission.*

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|------------------------------|--------|----|---|
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| <i>Idem</i> | (1933) | .. | The Therapeutics of Malaria: Principles of Treatment based on the Results of Controlled Experiments. Third General Report of the Malaria Commission. <i>Quart. Bull. Health Org.</i> , 2 , 2, pp. 181-285. |
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| SWELLENGREBEL, N. H. | (1933) | .. | Paludisme dans les Deltas. Delta du Rhin. <i>League of Nations</i> , C.H./Malaria/206, pp. 1-32. |
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THE STANDARDIZATION OF MIXED PREPARATIONS OF THE CINCHONA ALKALOIDS IN RELATION TO INDIAN CONDITIONS.*

BY

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(*Indian Research Fund Association.*)

[13th December, 1933.]

THE Malaria Commission of the League of Nations in their meetings in 1929-1931 studied carefully the information available as to the relative values of the different crystallizable alkaloids of cinchona bark. As a result of their deliberations, it was decided to recommend that the present mixed preparations of these alkaloids (cinchona febrifuge, total alkaloids, quinetum, etc.) should be replaced as far as possible by a standardized preparation to be known as 'Totaquina'. The composition suggested for this new product was as follows :—

'This preparation should contain at least 70 per cent of crystalline alkaloids, of which not less than 15 per cent must be quinine. Amorphous alkaloids should not, however, exceed 20 per cent, mineral matter 5 per cent and water 5 per cent'.

In this age of scientific medicine, the standardization of the contents of any drug used in human therapy is undoubtedly a proper procedure. It seemed reasonable, therefore, that products of such varied composition as the preparations of the mixed cinchona alkaloids should be brought into line with other therapeutic preparations, if they were to take their places in the Pharmacopœia of the world, either as substitutes for quinine or as additional weapons in the fight against malaria.

Large amounts of cinchona febrifuge are manufactured by the Government Cinchona Factories in India and the drug is widely used in that country. It is therefore necessary to consider carefully, in relation to malarial conditions in India,—

(a) the reasons for the suggested displacement of cinchona febrifuge and quinetum by a standardized preparation of the nature of totaquina;

* This note was prepared for the summer meeting of the Study Committee of the Malaria Commission of the Health Organisation of the League of Nations in 1933. It was circulated by that Organisation under its communication No. C.H./Malaria/201, dated 23rd March, 1933. A few minor alterations and explanatory foot-notes have since been added.

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(b) the uses to which it is proposed to put the new drug;

(c) the possibility of preparing a cheap totaquina;

(d) the material benefits which such a change might confer on the malarious population of India.

If, from a consideration of these various factors, it be definitely decided that the change from cinchona febrifuge to totaquina will confer a distinct *practical* benefit upon the malarious population of India, there is no doubt that such a change should be made.

REASONS FOR THE ADOPTION OF TOTAQUINA.

It is an axiom well known to all malariologists, that the treatment of the malarious sick should be the first step in any antimalarial campaign. This step is irrespective of any other measures initiated. There are not, however, enough antimalarial drugs produced annually in the world to treat more than a tithe of the people who suffer from the disease. On the other hand, the world stocks of the main antimalarial drug, quinine, have been accumulating steadily, partly because of the difficulties encountered in placing it in the hands of the sufferers, and partly because Governments cannot afford to purchase the amounts needed to treat all their sick. Malarious countries are usually poor and the cost of even a limited 'mass-treatment' campaign is often prohibitive, if quinine be the drug of choice for such measures.

The Malaria Commission of the League of Nations considered carefully the various means whereby the prohibitive cost of antimalarial drugs could be lessened, and the supply of antimalarial drugs increased, with resultant benefit to the poorer sufferers in malarious countries.

The evidence at present available goes to show that all the crystallizable alkaloids of cinchona bark have a definite therapeutic effect on the clinical manifestations of malarial disease. Although there may be some difference of opinion as to which of these alkaloids has the most powerful therapeutic action, there can be no doubt that the crude mixture of these alkaloids obtained from cinchona bark is a very useful drug in malarial therapy.

The Malaria Commission having considered the available evidence, decided that this was sufficient to show that some of the cheaper preparations containing all the crystallizable cinchona alkaloids, would give satisfactory clinical results. They therefore recommended the use of these mixtures for more widespread treatment, more especially in countries which could not afford to buy other antimalarial drugs in the very large amounts necessary to treat all their sick.

Having arrived at this conclusion, analyses of the different alkaloidal mixtures at present in use, showed that these had a very varied composition. It was therefore decided that a mixed alkaloidal preparation with a standard composition (*vide supra*), should be recommended for adoption by the National Pharmacopœia of different countries.

* The term 'mass-treatment' is not used in this paper to denote a wholesale and systematic treatment of all persons infected with malaria. It is intended primarily to mean the provision of treatment for patients who are unable to afford expert medical advice and supervision. This includes that large mass of patients who, especially in the tropics, suffer from attacks of the disease and are unable to obtain specific treatment for such attacks. The provision of such treatment has been hampered in the past by (a) the financial inability of many Governments to provide an adequate supply of suitable drugs, either free or at a cost within the financial means of poorer populations, and (b) the absence of facilities for the distribution of such drugs, when these are available.

There is no doubt that the adoption of such a standardized drug would be very valuable, by

(a) preventing the sale of markedly adulterated preparations of the cinchona alkaloids or those with little therapeutic value;

(b) maintaining a standard content of the effective alkaloids, and a diminution of those which appear to be of little therapeutic value, or even harmful in character;

(c) ensuring that therapeutic results obtained by such mixed alkaloids would be relatively constant, and would also be comparable from a scientific point of view; and

(d) providing a safe, reliable and cheaper drug in the treatment of malaria, for wider use in those countries which cannot afford quinine or other remedies in sufficient quantities to treat all their sick.

USES PROPOSED FOR TOTAQUINA.

It was not proposed that this new standardized preparation should displace quinine or other drugs of proved value in the treatment of malaria. The intention was that a good and cheaper treatment would be made available to supplement the supply of these drugs. If this can be done, it should be possible to make great extensions in treatment campaigns, in many countries, whose activities in this respect are now hampered by lack of funds. In the tropics it is probable that at the present time, not more than one-tenth of sufferers from malaria ever receive any specific treatment.

In many tropical countries widespread antimalarial measures are financially impossible and the chances of reinfection are high. The best results that can at present be hoped for from treatment under such circumstances, are that the clinical manifestations of attacks of the disease will be alleviated and the lives of the patients saved, in the majority of cases. It is, therefore, necessary to discuss in how far totaquina or other mixed alkaloidal preparations will meet the requirements of such a situation.

The properties which it is considered that any drug should possess for the ideal treatment of malaria have been detailed elsewhere (Sinton, 1930). These factors must therefore be discussed in so far as they affect mass-treatment with totaquina.

(a) So far as can be judged from the evidence available, such a mixture may possibly be slightly slower in its action, but for all practical purposes should be very little inferior to quinine, in controlling the clinical manifestations of the disease.* Such a cure of clinical symptoms is what the patient demands primarily.

(b) Many years of experience have shown that the cinchona alkaloids can be safely placed in the hands of the lay public for medical purposes, even in the absence of expert supervision.

(c) It has been the experience of most workers in the tropics that the uneducated, and often the educated, patient will stop taking any of the cinchona alkaloids as soon as clinical symptoms have disappeared, unless compelled to continue the treatment. Apart from the question of reinfection,

*This applies solely to the oral administration of the drug, which is the only method applicable under the conditions of mass-treatment for which totaquina would be most widely used.

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it cannot be hoped with treatments of such short duration, to produce a permanent cure in any large percentage of patients, whether quinine, cinchona febrifuge or totaquina is used.* For the cure of clinical symptoms in at least the less severe forms of the disease, preparations of the mixed alkaloids would seem to be almost as effective from a practical point of view as quinine.

(d) While the cinchona alkaloids will destroy the gametocytes of *P. vivax* and *P. malariae*, they have little effect on those of *P. falciparum*, usually the commonest parasite in the tropics. No one alkaloid has been proved markedly more effective in this respect than any other. It is here that drugs like plasmoquine have proved to be superior to the cinchona alkaloids.

(e) There is some evidence that quinine may have a greater effect in the production of a permanent cure in malignant tertian malaria than in the other infections. There is, however, no conclusive evidence that, when taken over the short periods mentioned above, the results will be markedly more successful than with the mixed alkaloidal preparations. The standard of 15 per cent recommended for totaquina, however, ensures that a considerable amount of quinine will be present in this drug.

(f) Additional factors in relationship of alkaloidal mixtures to mass treatment:—(i) They are cheaper in price than quinine. (ii) With some samples of the mixed alkaloids the taste is said to be slightly more disagreeable than that of quinine, and symptoms, such as nausea and vomiting, more common. These unpleasant effects are probably due to the content of amorphous alkaloids, about the therapeutic value of which considerable doubt exists. In the standardized preparation it has therefore been laid down that such amorphous alkaloids should not exceed 20 per cent. (iii) They require little

* The recent investigations of Mulligan and Sinton (1933, 1933a) on monkey malaria, suggest that, if a patient can be tided over the acute primary attack of a malarial infection and the earlier acute relapses by means of treatment, he will develop a considerable degree of tolerance to the clinical manifestations of the strain of parasite causing the disease. Later superinfections with the same strain produce little or no exacerbation of the symptoms, so long as a latent infection is present. Superinfection with a heterologous strain of parasite may, however, produce an acute attack almost equal in severity to the primary one, but, if this be tided over by renewed treatment, a tolerance to this strain is also developed.

It appears probable that, in hyperendemic areas where infection and superinfection are constantly taking place, with each successive year of life, children gradually develop a tolerance to a greater and greater number of strains of the local species of *Plasmodium*, if they survive the acute attacks caused by such infections. When they reach adolescence, or even before that, they have probably developed a tolerance to all or most of the local strains of parasite. This is a possible explanation of the tolerance to malarial infection shown by the adults in such areas. This tolerance may, however, be broken down under exceptional circumstances such as temporary conditions of lowered immunity, the introduction of a foreign strain of parasite, or a very large increase in the infecting dosage, i.e., quantum of sporozoites, or possibly an increase in the virulence of one of the local strains of parasite.

The production of a permanent cure of one infection, under such conditions of multiple reinfection, seems useless and even inadvisable. On the other hand, the important factor seems to be the provision of treatment to tide the patient over the dangerous periods of the acute attacks, and thus enable him to develop the tolerance which is so useful to him in adult life.

Under conditions where the chances of reinfection are slight, due either to the mildly endemic nature of the malaria, or to the measures taken to prevent the disease, the possible production of a permanent cure appears more important. In such areas uncured cases may act as foci of infection, thus tending to cause an increase in malaria. Such patients in the absence of reinfection tend to spontaneous cure and thus lose much of the tolerance, which in hyperendemic areas is kept at a high level by the vaccination of repeated reinfection with the same strains.

expert supervision. (iv) As noted previously, it cannot be hoped that totaquina will produce a permanent cure in a few days in the majority of patients, but in this respect it is probably little inferior to quinine.

Several of the newer drugs, such as plasmoquine and atebirin, fulfil some of the conditions of an ideal treatment where the cinchona alkaloids fail, but even these drugs do not fulfil all the conditions. For the present they can only be looked upon as adjuvants, but very important ones, to treatment with the cinchona alkaloids. Unfortunately the cost of these drugs, and in some cases the very narrow limit between their therapeutic and toxic doses, make them unsuitable for mass-treatment, except under very special conditions of medical supervision, which are seldom available in most areas in the tropics.

From the data given above, it would appear that a mixed alkaloidal preparation of the nature of totaquina, would probably be little, if at all, less valuable than quinine for mass-treatment, as carried out in the tropics.

THE PREPARATION OF A CHEAP TOTAQUINA.

EXTENSIONS OF CINCHONA CULTIVATION

Mixtures of cinchona alkaloids have been in use for many years in the form of substances such as cinchona febrifuge, residual alkaloids, quinetum, etc. The price of such drugs is less than that of quinine, because the method of manufacture is less costly and because, in some instances, they are the by-products of crude mixtures from which quantities of the more fashionable and expensive alkaloid, quinine, have already been extracted. The cost is also lower, because the bark employed for their manufacture is sometimes one which has a low quinine content and is, therefore, less profitable to use for the extraction of the latter drug.

The original Indian cinchona febrifuge, quinetum and quinum were mixtures of the total alkaloids from the bark of species of *Cinchona* like *C. succirubra*.

The present Indian cinchona febrifuge is a mixture of the residual alkaloids, left after most of the quinine has been extracted from the quinine-rich bark of *C. ledgeriana*, and to which residue sufficient quinine has been added to make its composition very similar to that of the original febrifuge. This change was necessitated by the scarcity of *C. succirubra* bark following upon the abandonment of cultivation of this tree by private planters in India. This was due to the marked fall in price of quinine, resulting from the great success which followed the cultivation of *C. ledgeriana* in Java.

The high price of quinine depends largely upon the fact that the tree which gives the highest yield, *C. ledgeriana*, will only flourish under very special conditions of climate and soil, which are only found in very limited areas. Although the hardier species, like *C. succirubra* and *C. robusta*, will flourish over much wider areas, their yield of quinine is too small to make their cultivation a commercial proposition, if, as at present, quinine remains the chief drug to be used for malarial treatment. With the wider adoption of the other alkaloids in the treatment of malaria, the cultivation of such trees might again become commercially profitable for the production of the cheaper drug totaquina. The hardier nature of these trees might even make it possible for most of the malarious countries in the tropics to produce their own supplies of this drug at a low cost, or at least to supplement their present supplies of antimalarial drugs. This eventuality was considered when the Malaria Commission fixed the standard for totaquina at approximately the total alkaloidal content of the bark of such trees.

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If bark of this nature were now available in sufficient amounts, the cost of totaquina should remain low. Unfortunately, it seems that the supply of such cheap bark is at present small, as judged by the potential needs of such a widespread campaign as is suggested. When this is exhausted the demand for totaquina will have to be met chiefly by the use of by-products from the *ledgeriana* bark used to produce quinine, until such time as new bark becomes available. Under the latter circumstances, it seems doubtful whether a totaquina of the high standard suggested can continue to be produced at the low price anticipated.

To prevent such a rise in price of totaquina, it would be necessary to extend very widely the cultivation of the hardier cinchona trees to meet the demand for cheap bark. The object of such plantations should *not* be to *supplant* those cultivated for the production of quinine, *but to supplement* the supply of antimalarial drugs for the needs of the poorer sufferers of malarious countries.

It must be remembered, however, that such new plantations will not yield bark for a considerable number of years, sometimes 10 or more. In the meantime it seems possible, as suggested above, that the price of mixed alkaloidal products of the standard of totaquina will rise, if there be a great demand for this substance as is anticipated.

While from a medical and agricultural point of view the extension of cultivation of the hardier species of cinchona, seems a most laudable object, the policy apparently involves much wider issues than these. Such a change in policy might almost be compared to a departure from the gold (quinine) standard.

Various questions will need to be discussed fully and carefully before such a step is taken.

(a) Will some new synthetic drug be discovered in the near future which will be cheaper and equally efficacious for the use envisaged for totaquina?

(b) Will it be possible to meet the demand for totaquina in the near future at the present low price, taking into consideration the supply of cheap bark available?

(c) What effect will the increased demand for the other alkaloids have on their price as compared with quinine?*

(d) Will a fall in the price of quinine make such new plantations commercially unsound or unnecessary in the future?

POSSIBLE EFFECTS OF STANDARDIZATION ON THE PRESENT PRICE OF INDIAN CINCHONA FEBRIFUGE

There is no doubt that the cinchona febrifuge used in India may vary very considerably from time to time, in the relative proportions of the different alkaloids present. That such a variation should occur, is not to be wondered at, when one takes into account the fact that these alkaloidal mixtures are mainly manufactured as a by-product in the extraction of quinine from cinchona bark of very diverse alkaloidal content. Under such conditions some standardization of the mixed alkaloidal product would seem essential.

* The Madras Commission of 1866-1867 reported that certain of the other alkaloids of cinchona bark were as good as quinine for the treatment of malaria. The result was that the price of these alkaloids immediately rose to be equal or even greater than that of quinine.

When, however, one considers that the low cost of cinchona febrifuge in the past has depended to some extent on the absence of any complicated process of standardization, one is led to enquire whether such a standardized product could be manufactured without a considerable increase in cost. Such an increase, if considerable, might neutralize largely the value of this drug as compared with quinine, *i.e.*, the difference in price.

From the information available at present, it would appear that a change in the present system of manufacture of cinchona febrifuge, so that it should be replaced by totaquina, would result in an increase in the cost of production. The preparation of such a standardized mixture would require more complicated process, more elaborate analyses* and a greater addition of some of the more expensive crystallizable alkaloids, if, as at present, it is only manufactured as a by-product in the extraction of quinine. It is impossible to say at the moment how much this extra cost will be eventually, or how far such an increase in price would annul the advantages of the present low cost of such mixed alkaloids.†

All these points would require very careful consideration by experts, medical, financial, and agricultural, before any extensive changes were made in the present methods of manufacture of mixed alkaloidal products in India.

THE BENEFITS ARISING FROM THE REPLACEMENT OF CINCHONA FEBRIFUGE BY TOTAQUINA UNDER INDIAN CONDITIONS.

At present most of the cinchona febrifuge and similar alkaloidal mixtures used in India are manufactured by government departments. Under such circumstances, there seems little likelihood that these products will fall much below their present total crystallizable alkaloidal content. If, however, the manufacture of such products were to be taken up by commercial firms in India on a large scale, or large amounts of these drugs were imported from very varied sources, the need for a standard composition would at once be urgent.

Such a standardization of the Indian cinchona febrifuge would result as a rule in an increase in the amount of crystallizable alkaloids and a diminution in the percentage of the amorphous ones.‡ The former change would probably be beneficial by making some increases in the rapidity of clinical cure, but this benefit might also be obtained by a slight increase in dosage. The decrease in the percentage of amorphous alkaloids would probably be advantageous causing a diminution in the nauseating effects produced by these alkaloids. These effects would also tend to be augmented by any increase in the dosage as suggested above, so some diminution in the latter alkaloids seems essential.

For the reasons mentioned, the presence of a high content of crystallizable alkaloids combined with a low one of amorphous alkaloids, would seem to be the objective to be aimed at in any standardized product for mass distribution.

* There also seems to be no very close agreement between the analyses of this substance made by various expert quinologists, even in respect of the points upon which the Malaria Commission has laid stress.

† Since this note was originally written, the Cinchona Department of the Madras Government have prepared a 'totaquina' according to the standards laid down by the Malaria Commission. In a recent communication they have informed me that the total costs of production (ex-factory) of cinchona febrifuge, totaquina and quinine sulphate, are in the proportions of about 1, 2, and 3 respectively.

‡ Vide analyses collected by Sinton (1930a).

12 *Standardization of Mixed Preparations of Cinchona Alkaloids.*

Even if at present a product of the standard of totaquina could not be produced in India, at a price which would maintain its practical value in comparison with quinine, it might be possible to remedy some of the defects of cinchona febrifuge to a considerable extent, without materially affecting its cost.

From a consideration of the probable therapeutic effects of quinine and cinchona febrifuge when used for mass-treatments, it seems doubtful whether totaquina would have any very extraordinary advantage over such an improved cinchona febrifuge manufactured in the Government factories in India.

If the price of such a preparation should turn out to be much greater than that of the present cinchona febrifuge, it seems possible that more beneficial results to the population might be obtained at the same cost by a wider use of the latter product than a more limited one of the former. Even a rise of half an anna per patient per annum in the cost of treatment of each individual among all the estimated 200 millions, who suffer from malaria each year in India, would mean a total sum of nearly half a million pounds sterling. It is not a question of what is the best drug procurable, but of what drug will give the greatest benefit to the greatest number for the money available in poor countries.

The necessity for the standardization of a mixed alkaloidal preparation seems to me a question which is at the moment of much more urgent import under European conditions than in a country like India, which manufactures its own product.

In Europe (i) the facilities for a widespread distribution of treatment are much greater than in India or other tropical countries; (ii) the chances of reinfection after cure are usually less, and many more people will continue their medicine after clinical symptoms have abated, so it is necessary to have as effective a drug as possible to increase the chances of permanent cure; (iii) the actual number of malarial cases is small, compared with the millions in India, so slight differences in cost between cinchona febrifuge and totaquina would not be so important; and (iv) countries buying drugs of such variable composition from a large number of different sources, must necessarily insist upon a standard composition.

In India on the other hand, the most urgent problems of malarial treatment which confront the Government at the moment are not the standardization of cinchona febrifuge, but (i) how can the present available supply of antimalarial drugs be used to produce the greatest benefit to the greatest number of sick; (ii) how can these drugs be most easily and cheaply placed within the reach (both financial and physical) of the afflicted population; (iii) how can such a distribution of these drugs be continued and extended in future; and (iv) how can the cost of these drugs be reduced?

Under such circumstances it would seem that, however desirable the production of a standardized preparation of the mixed cinchona alkaloids may be, it is not of such urgent importance to India at the moment, as it is to many other countries.

While admitting the importance of a standardized preparation, this question should not be allowed to assume such proportions that it obscures the other more immediately urgent problems relating to wider facilities for treatment among the malarious masses in India.

As the question of increase in the area of cinchona cultivation must necessarily be settled many years before the demand for the bark comes, this

problem would need to be settled in the near future. This does not, however, seem possible until many of the points raised in this note are settled by a conference thoroughly familiar with the very varied aspects of the problem.

CONCLUSIONS.

1. A standardization of the alkaloidal contents of mixed alkaloidal preparations of cinchona bark is to be encouraged.

2. Such preparations seem eminently suitable for mass-treatment among malarious populations who cannot afford more expensive drugs and who cannot easily obtain medical advice.

3. Before the manufacture of the present cheap alkaloidal mixtures are replaced by that of such standardized preparations, it is necessary to consider carefully (i) what effect this will have on the present cost of such products; (ii) what effect it will have on the present policy of cinchona cultivation; and (iii) how many years it will take to grow sufficient bark from such hardy trees as *C. succirubra* and *C. robusta*, to meet the demand for bark to manufacture a cheap and adequate supply of a total alkaloid preparation of the character mentioned.

4. Serious attempts should be made to improve the Indian cinchona febrifuge by an increase in total crystallizable alkaloids and a diminution in the amorphous ones, if this can be done without any very great increase in cost.

5. The more urgent problems, which at the moment confront India in connection with the treatment of her large malarious population, are more closely allied with the provision of means for the distribution of treatment to the malarious masses of the country, rather than the standardization of cinchona febrifuge.

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ERRATA.

Page 38, Table B, under 'Hæmozoin', line 2nd, insert 'Λ' after 'between' and before '='.
 Inside of back page of Cover, Vol. III, No. 3, line 6th, substitute 'Spain' instead of 'Assam' in 'Rice Cultivation in Assam'.

STUDIES OF MALARIAL PIGMENT (HÆMOZOIN).

Part I.

INVESTIGATION OF THE ACTION OF SOLVENTS ON HÆMOZOIN AND THE SPECTROSCOPICAL APPEARANCES OBSERVED IN THE SOLUTIONS.

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I. INTRODUCTION.

THE accumulation of black material in the liver and spleen was considered to be a very important point in the humoral pathology of the ancient physicians. From the time of Galen almost up to the middle of the 19th century, the causation of many diseases was believed to be associated with 'black bile'.

The occurrence at autopsy of dark coloration in some of the internal organs was recorded by several of the older writers. It was noticed more especially in connection with the intermittent and remittent fevers, and the so-called marsh fevers.

The dark coloration of such organs as the liver, spleen and brain was noted among others by Lancisi (1717), by Stoll (1797), by Folchi, by Montfalcon (1824), by Bailly (1825), by Billard (1825), by Popken (1827), by Fricke (1827), by Chisholm, by Annesley (1828), by Bright (1831), by Twining (1832), by Maillot (1836), by Stewardson (1841), and by Haspal (1850).*

At that period, although morbid anatomy had made considerable advances, morbid histology was still in its infancy. The causation of the dark coloration was not suspected until Meckel in 1847 observed the presence of pigmented bodies in the blood and spleen during the post-mortem examination of an insane patient. He did not, however, seem to have connected these findings with malaria. Dlauhy in Vienna, at almost the same time, found similar bodies during the autopsy of a case, which had died suddenly with 'typhus-like' symptoms. The latter worker demonstrated the condition to Virchow, who shortly afterwards observed similar structures in the blood and spleen of a patient who had died of chronic malaria (Virchow, 1849).

Virchow recognised the importance of these pigmented bodies in relation to malaria, and later published illustrations of them (Virchow, 1858). These observations were quickly confirmed by Heschl (1850, 1862), by Planer (1854), by Clark (1855), by Frerichs (1855) and by Charcot (1857). The close relationship between the pigmentation and the intermittent and remittent fevers was soon widely recognised, although many observers thought that pigmentation of a similar nature might also occur with other diseases. This mistake appears to have been largely due to the confusion of hæmosiderin and other hæmatogenous pigments with malarial pigment.

* For a more detailed summary of the earlier observations on such pigmentation, the reader is referred to the works of Frerichs (1858), of Marchiafava and Celli (1884), of Laveran (1891, 1907), of Mannaberg (1893, 1906), of Welch (1897), of Marchiafava and Bignami (1900) and of Thayer (1900).

On account of the abundance of pigment in the spleen, Meckel (1847) and Virchow (1849) considered this organ to be the chief site of origin of the substance. Planer (1854) was the first to record the presence of pigmented cells in the blood of a living patient. He thought, however, that the pigment originated in the blood stream and that the pigmentation seen in the internal organs was secondary to this.

These two views gave rise to considerable discussion during the next 30 years.

The theory of splenic origin, with possibly a minor hepatic focus, was warmly supported by Frerichs (1855), by Charcot (1857), by Grohe (1861), and by Mosler (1874, 1877). On the other hand, the hæmatogenous view of Planer (1854) was strongly advocated by Arnstein (1874) and by Kelsch (1875, 1876).

The exact mode of origin of the pigment was not explained by any of these workers although many of them thought it might arise from the blood, either directly or indirectly. Kelsch (1876) suggested that the destruction of the red cells caused a saturation of the blood with the pigment in solution, and that, when this was precipitated, it was taken up by the leucocytes. Marchiafava (1879) believed in the blood origin of the pigment, and suspected that it might be formed inside the red cells. This hypothesis was elaborated by Marchiafava and Celli (1884), who thought it might arise as the result of a 'necrobiosis' of the red cells.*

There is little doubt that several of the earlier workers had seen the malaria parasite without realising its parasitic nature. Kelsch (1880) concluded that pigmented leucocytes were characteristic of malaria and that their presence was diagnostic of this disease. It was only with the discovery of the malaria Plasmodium by Laveran in 1880 that the true nature of the pigment, and of the dark coloration of the internal organs, began to be recognised.

II. PREVIOUS WORK ON THE NATURE OF MALARIAL PIGMENT.

(a) THEORIES AS TO THE MODE OF ORIGIN OF THE PIGMENT.

With the discovery of the malaria parasite, new ideas as to the mode of origin of the pigment naturally arose. Most workers now consider that it is formed by the digestive action of the Plasmodium upon the hæmoglobin of the infested red cells. During this process it is believed that the protein portion (globin) of the hæmoglobin is used as nutriment by the parasite, while the pigment portion (hæmin) remains as a waste product. Brown (1911) suggests that the parasite has an enzyme-like pepsin or trypsin, which splits the hæmoglobin into these parts. A similar action has been suggested by other workers. Seyfarth (1926) thinks such a lytic action digests the globin and leaves the rest of the hæmoglobin free (hæmochromogen) as the crystalline malarial pigment.

Several workers have expressed other views as to the mode of origin of the pigment. Danilewsky (1896) suggested that the pigment is formed inside the parasite at the expense of the paranuclein, because there was no evidence that hæmoglobin could be transformed directly into malarial pigment. Comes (1922) thinks that the pigment of *P. vivax* and *P. malariae* is formed in the 'nutritive vacuole' of the parasites, under the influence of the adjacent nucleus. For this reason it is first observed in the region of the 'vacuole'. In the case of the sexual forms of *P. falciparum*, he considers the nucleus produces the

* For a more detailed account of this controversy, the reader is referred to Marchiafava and Celli (1884), to Laveran (1891, 1907), to Mannaberg (1893, 1905), to Welch (1897) and to Thayer (1900).

pigment without any intermediary function of the 'vacuole'.* He does not, however, deny the origin of the pigment from hæmoglobin. The fact, noted even by very early observers, that pigment granules are not seen inside the 'vacuole', does not support the suggestion made by Comes.

Warasi (1928) points out that *P. malariae* produces more pigment than does *P. vivax*, yet it 'dehæmoglobinises' the infested red cell to a less extent. He, therefore, argues that, if the pigment be merely the result of the fermentative katabolism of hæmoglobin, different proportions of pigment would be produced by the two parasites, i.e., proportionate to the supposed amounts of hæmoglobin used up. He thinks on this account that the malaria parasite builds its pigment from assimilated material, or from the permanent portions of its own body, and not entirely from the hæmoglobin of the red cell.

Variations in staining† and other optical appearances of infested erythrocytes may possibly indicate changes in the amounts of hæmoglobin present in such cells. It appears to us, however, that these variations cannot be considered as a quantitative measure of the amount of this substance present. We know of no experimental evidence to support the scientific use of the term 'dehæmoglobinisation' in respect of the pallor of infested red cells.† The enlargement of the infested cell with *P. vivax* as compared with *P. malariae* might be an explanation of lighter colour in the former cells, i.e., the hæmoglobin is spread in a thinner layer. It is also possible that the stippling seen with infections of *P. vivax* and other Plasmodia may be due to local precipitations of hæmoglobin or some of its derivatives. This would account for the greater general pallor of cells infested with *P. vivax* as compared with *P. malariae*. Apart from any other possible explanations of the differences in the amounts of pigment, which may be simply an inherent character in different species of *Plasmodium*, one would naturally expect a larger amount of pigment with *P. malariae*, because of the longer duration of its vegetative cycle in the human host. The amount of pigment in the longer-lived sexual forms is also greater than in the asexual parasites.

The fact, recorded later in this paper, that solutions of hæmozoin show the spectroscopical appearances of hæmatin, denotes the origin of malarial pigment from hæmoglobin, for this is one of the most delicate tests for the derivatives of the latter pigment.

(b) THEORIES AS TO THE NATURE OF MALARIAL PIGMENT.

Two main theories have been formulated as to the nature of malarial pigment. The first and older theory was that it was similar in character to 'melanin', the black pigment found in the skin and some other tissues. The second and more modern view is that it is a hæmatogenous pigment closely allied to hæmatin.

* If, as believed by many observers, the 'vacuole' be a portion of the true nucleus of the malaria parasite, the suggested relationship of pigment formation to the nucleus of the parasite would be somewhat similar in the three species of *Plasmodium*. The view of Comes (1922) would then resemble that put forward by Danilewsky (1896).

The fact that the zymogens of cells have been found to appear in the neighbourhood of the nucleus, suggests that the enzyme which transforms hæmoglobin to malarial pigment is derived from this structure.

† Although it is known that eosin and some other stains have a great affinity for hæmoglobin, this reaction is not specific.

(1) The melanin theory.

Meckel (1847) appears to have been the first worker to use the term 'melanin' to describe malarial pigment. During the next 60 years the pigment was considered to be closely allied to, if not identical with, this substance. For this reason the older writers described the conditions of the blood and of the tissues associated with the presence of malarial pigment, as 'melanæmia' and 'melanosis' respectively.

Ross (1911) suggested the name 'plasmodin' for this substance. Askanazy (1919) called the pigment 'hæmo-melanin' to distinguish it from true melanin, the black pigment of the tissues, such as the skin, the eye and certain tumours. The latter substance he named 'histo-melanin'. The name 'hæmozoin' was proposed by Sambon, and is now the term in common use in English literature.

Brown (1911) undertook an exhaustive research into the properties of hæmozoin, and compared these with those of true or tissue melanin. He found many definite chemical and physical differences between the two substances. Hueck (1921) also records other differences between true melanin and hæmozoin.

Brown (1911) reports that, in contradistinction to hæmozoin, true melanin is not soluble in a saturated solution of lithium carbonate, in 0·2 per cent aqueous solution of KOH, in strong ammonia, in alkaline or acid solutions of alcohol, nor in ammonium sulphide. The bleaching effects of oxidizing agents such as KMnO_4 and H_2O_2 do not coincide in the case of the two pigments. Hueck (1921) also states that malaria pigment is soluble in acid alcohol, but it is not acted upon by osmic acid and does not give any silver reduction reaction while the reverse is true of melanin.

Nowadays, by the term 'melanin' is understood in pathological anatomy, or rather chemistry, a relatively well delimited group of pigments, which, because of their chemical properties and more especially of their decomposition products, occupy a quite separate position (Glasunow, 1925). These substances are usually devoid of iron, while hæmatin contains this metal.

Many workers in the last 30 years have made extensive studies of malarial pigment and their results confirm the view that this substance is not identical with true melanin. It has now been generally accepted that this is so. Warasi (1927), however, still considers that hæmozoin is more closely allied to an iron-containing melanin than to hæmatin.

(2) The hæmatin theory.

The probable origin of hæmozoin from hæmoglobin naturally suggested that iron might also be present in the former substance. None of the earlier workers, using the Berlin-blue reaction of Perls (1867)*, were able to demonstrate the presence of this element microchemically. Later investigations with the Turnbull-blue reaction* have also given negative results. The absence of iron, demonstrable by these methods, is considered to be one of the characteristics of malarial pigment.

It must be remembered, however, that neither of these reactions will reveal iron, if it be not present in an ionisable form. If the metal be present as

* In the Berlin- or Prussian-blue reaction, a blue colour is produced when potassium ferrocyanide and hydrochloric acid, in the concentrated form or in aqueous solution, are allowed to act on ferric iron. With the Turnbull-blue reaction, ferricyanide is substituted for the ferrocyanide and a blue colour is produced with ferrous salts.

(i) That the action of certain reagents used for fixation may cause considerable alterations in hæmoglobin or its derivatives, is a well known phenomenon. The most usual of these is the action of formalin, which some workers believe produces a pigment with many characters in common with hæmozoin.

(ii) Even assuming that the pigment studied by previous workers had its origin in the malaria parasite, there is no proof that it had not undergone some change in composition after phagocytosis. The fact that hæmozoin tends to disappear from the body after a malarial infection is permanently cured, suggests that some change in its composition may take place in the tissues.* Indeed Mayer (1923) reports that granules of pigment, seen in the macrophages of sections of spleen and bone-marrow, gave a 'positive Berlin-blue reaction, when treated with a special technique. On the other hand, he was unable to demonstrate this reaction with the pigment of endo-globular parasites lying in red cells adjacent to the same macrophages. This observation suggests that the cells of the reticulo-endothelial system may have an action on the pigment, probably by virtue of enzymes. Lubarsch (1925) thinks, however, that the phagocytosed pigment seen by Mayer may have been hæmosiderin and not hæmozoin.

(iii) It is well known that hæmatogenous pigments may accumulate in the spleen and other organs in many diseases apart from malaria. These pigments, such as hæmosiderin, hæmofuchsin, hæmotoidin, etc., occur most commonly under conditions of extensive destruction of the red blood cells and this also occurs in malaria. Although it is now recognised that the properties of these pigments are different from hæmozoin, yet there is no doubt that they were confused with it by several of the older workers (cf. Ewing, 1903, 1905).

Schumm (1913, 1916) records the presence of hæmatin in the blood in malaria. This finding supports the view that hæmozoin may be identical with hæmatin. On the other hand, Butterfield and Benedict (1914) were unable to demonstrate the latter pigment in the blood of 19 cases of malaria showing parasites in the blood. Several workers have also reported that hæmatin may occur in the blood under various other conditions of excessive blood destruction.

Feigl, Knack and Koopmann (1916) and Feigl (1917, 1919) found it in conditions of over-exertion, such as forced marches. In pernicious anæmia it has been recorded by Schumm (1912, 1916) and by Bingold (1926). It was reported in acute toxic conditions, due to certain organic and inorganic poisons, by Schumm (1912, 1916), Feigl (1916, 1918, 1919) and Bingold (1926). It has also been found in acute yellow atrophy of the liver, in eclampsia, in congenital hæmatoporphyrinuria and acute toxic hæmolysis. Feigl and Deussing (1918) report the pathological occurrence of hæmatin-æmia in 700 cases.

The occurrence of hæmatin in the blood in many conditions other than malaria would appear to indicate that conclusive evidence as to the close relationship of this pigment to hæmozoin cannot be obtained from a study of tissue pigments only.

It is evident from a consideration of the factors discussed above that conclusions, based on the study of tissue pigments only, are open to several possible fallacies. These can only be eliminated by a study of fresh parasitic pigment. To overcome these difficulties our investigations have been carried out, unless otherwise stated, with pigment isolated from fresh, heavily parasitised blood.

*Urriola (1911, 1924) suggested that malarial pigment is excreted in the urine in a granular form. Steinfeld (1926) was, however, unable to find any pigment granules in the urine of malarial patients, which could be recognised as hæmozoin with certainty.

(iv) The possibility of changes in the pigment, due to the action of the solvent used to extract it from either the tissues or the parasites, has been investigated. The results of this work are reported in Section IV (c) of this paper.

III. THE ISOLATION OF LARGE AMOUNTS OF MALARIAL PIGMENT FROM THE BLOOD.

Pigment extracted from the spleen, liver or brain of human cases of malaria, has formed the basis of all the more detailed studies of hæmozoin made in the past. While it is comparatively easy to obtain an abundance of such tissue, it is extremely difficult to get human malarial parasites in large quantities. To collect the relatively large amounts of pigment used in our work, we have relied upon blood from experimental infections in monkeys.

Plasmodium knowlesi Sinton and Mulligan, 1932, a natural parasite of *Silenus irus*, causes an extraordinarily massive blood infection when inoculated into a susceptible host such as *S. rhesus* (vide Sinton and Mulligan, 1933). These infections are almost invariably fatal if untreated. By bleeding such infected monkeys to death at the height of the infection it was possible to obtain large quantities of heavily parasitised blood.

TECHNIQUE OF ISOLATION OF PARASITES.

The following technique, devised by Sinton and Mulligan (1932), was used to obtain large amounts of a concentrated suspension of highly pigmented Plasmodia.

A young specimen of *S. rhesus* was infected with *P. knowlesi* by blood inoculation. The infection was allowed to proceed unchecked until the parasite count was very high (sometimes as much as 2,500,000 per c.mm.). When it was anticipated that the animal would not survive another segmentation of the parasites, hourly blood examinations were made to determine the period of maximum pigmentation, i.e., when the majority of parasites were mature schizonts but rupture had not commenced. The animal was then anaesthetised with chloroform and the thorax opened before the heart had stopped beating. Several 20 c.c. syringes, each charged with about 4 c.c. of citrate-saline solution,* were used to withdraw the blood from the heart. In this way it was possible to obtain as much as 80 c.c. of blood from a small monkey.

The citrated blood was centrifuged to separate the plasma from the cells. The former was pipetted off and the cells were again washed thrice in fresh citrate-saline solution to remove all traces of plasma. At each washing any detectable layer of leucocytes was removed from the surface of the deposit. The deposit in the tubes showed a very dark grey layer on top, composed mainly of red cells infested with heavily pigmented parasites. At the bottom was a bright reddish layer consisting chiefly of normal red cells, or those having only young parasites.

The dark layer was pipetted off and suspended in saline solution. The parasites were washed several times in the solution by centrifugation, and on each occasion the dark layer only was collected. The deposit eventually obtained, consisted of a very dark grey mass, which on microscopical examination was found to be composed of heavily parasitised erythrocytes with very few leucocytes.

The mass of parasitised cells was then mixed with distilled water, to bring into solution any hæmoglobin which might be present in the cells of the deposit. By thorough mixing and repeated washing with distilled water, followed by centrifugation, a dark grey gelatinous deposit was obtained with a clear supernatant fluid in which no hæmoglobin could be detected.

* The citrate-saline solution had the following composition:—sodium citrate 15 gms., sodium chloride 85 gms., and distilled water to 1,000 c.c.

This deposit of highly pigmented parasites, freed from all traces of hæmoglobin, was used as the material upon which our investigations were made. By this method it was possible to obtain large amounts of pigment, which was only parasitic in origin and which had not been altered by fixation or phagocytosis.

IV. THE ACTION OF SOLVENTS UPON HÆMOZOIN AND HÆMATIN.

Ever since the discovery of malarial pigment, a large amount of work has been done to determine the action of solvents upon this substance. The earlier investigations of the subject have been summarised by Welch (1897) as follows:—‘Since the examination of malarial pigment by Meckel and by Frerichs, it has been known that concentrated sulphuric and hydrochloric acids do not alter it, and that it disappears upon the addition of strong alkalies and chloride of lime. Kiener observed that the pigment was dissolved by ammonium sulphide’. As has been noted earlier in this paper, a large amount of further work has been done on hæmozoin since Welch wrote in 1897. Almost without exception, these researches were carried out with pigment obtained from fixed tissues, so it is necessary to compare the results recorded with those obtained with fresh pigment direct from malarial parasites.

In our work the effects of the different solvents were tested mainly upon suspensions of malarial parasites isolated by the method just described. Where, in describing in detail our different experiments, we have stated that ‘malarial pigment’ or ‘hæmozoin’ was used, these terms have been employed, for ease of reference, to denote such suspensions of isolated parasites, unless otherwise stated. No attempt was made, except for special experiments, to free the pigment from the remains of the parasites, as it was considered that such a procedure might cause a change in the character of the pigment, thus introducing a possible fallacy into our results. This point has been discussed in greater detail at a later stage of this paper.

Control experiments were carried out with hæmatin prepared from horse’s blood by the method described by Plimmer (1915).^{*} The results of a comparison of these two pigments have been given in Table A.

(a) INVESTIGATION OF THE ACTION OF SOLVENTS.

(1) Alkalies.

Most modern workers have reported that hæmozoin is soluble in aqueous solutions of alkalies [NaOH , KOH , $(\text{NH}_4)\text{OH}$], even when comparatively weak solutions are used. On the other hand, Mannaberg (1893, 1905), Thayer (1900), v. Limbeck (1901), Laveran (1907) and Craig (1909) speak of the action of strong alkalies as clearing up or changing the colour of the granules to reddish-brown and then to yellow.

In our experience with malarial pigment, both from isolated parasites and from fresh or fixed splenic tissue, such alkalies as KOH , NaOH or $(\text{NH}_4)\text{OH}$ dissolve hæmozoin, or more correctly speaking peptise it.

^{*} Hæmin was first prepared by the method of Schälfejeff. This substance was then dissolved in cold 10 per cent solution of NaOH , and to the resultant solution dilute HCl was carefully added. The precipitate of hæmatin formed was filtered off and washed.

Experiment (1).

The latter action was verified by examining solutions of pigment made in N/10 KOH and N/10 NaOH. Such pigment solutions were dialysed in parchment thimbles against the original solvent reagents. Even after 24 hours of dialysis, practically the whole of the pigment remained inside the thimble, while the solvent outside was almost colourless. Solutions of hæmatin, and of pigment isolated from parasites by papain digestion, behaved in a similar way. This shows that practically all the pigment is in a colloidal state and only a very small fraction in true solution, i.e., where the solute is present in a molecularly dispersed state.

The stages of 'solution' of the granules of hæmozoin were observed under the microscope. It was found that while the large pigment granules had a dark colour, as they became reduced in size and thickness by the action of the alkali solvent, their colour gradually became lighter. This difference in colour between thin and thick masses would account for the statements made by Mannaberg, Thayer, etc. Observations of the development of pigment inside malarial parasites also show that the very fine particles of pigment which are first visible, have a much lighter colour than the dark masses seen in the adult Plasmodia.

Wats and White (1932) report that, while the pigment studied by them was soluble in N/1 KOH and N/1 NaOH, it was not dissolved by N/20 NaOH. In view of the large number of workers who have found this pigment soluble in even weaker solutions of alkali, it would seem that the pigment studied by Wats and White (1932) was either not hæmozoin, or, if so, it had been altered by fixation of the tissues.

Like Brown (1911), Hueck (1921), Seyfarth (1926) and many other workers, we have found that hæmozoin is easily soluble in alcoholic solutions of NaOH and KOH, even in as weak dilutions as 0.04 per cent.

The action of acids on alkaline solutions of hæmozoin and of hæmatin.

Some interesting results were obtained when alkaline solutions of hæmozoin were acidified with a few drops of concentrated HCl. This results in the formation of acid hæmatin.

When the solution was derived from pigmented parasites, it was found that the acid hæmatin separated out from the solution and sedimented in about 4-5 hours. A similar precipitate obtained from an alkaline solution of pure hæmatin sedimented at once. On the other hand, if whole blood be diluted with water, treated similarly with acid and warmed, the acid hæmatin so formed remained in solution indefinitely. As the pigment extracted by us from pigmented parasites appeared to be hæmatin these differences required further elucidation.

It appeared probable that the acid hæmatin, produced from the blood in the above experiment, was not in a state of true solution, but present in a colloidal state. The protective action of the proteins of the blood appeared to have prevented the flocculation and subsequent sedimentation of the precipitate. Several experiments were carried out to test this hypothesis.

Experiment (2).

When a small quantity of gelatine was dissolved in the alkaline solution of parasite pigment, and the solution subsequently acidified, the acid hæmatin remained indefinitely in solution. This supports the view that proteins can protect a suspension of pigment against flocculation and precipitation in acid solution.

Experiment (3).

An aqueous suspension of isolated pigmented parasites was digested for a long period with papain and then centrifuged. The deposit was then washed several times with distilled water. It was hoped that in this way the digestive action of the enzyme would bring into solution most of the proteins of the parasites, and that these would be removed by the washing. When the resultant pigment was made into an alkaline solution and then acidified, the acid hæmatin precipitated almost immediately. So far as we were able to determine, this rapid sedimentation was not due to any detectable change in the pigment produced by the action of papain.

The amount of protein remaining with the pigment granules after the digestion must have been very small, and its protective action appears to have been negligible. If, however, a small amount of gelatine be added to the alkaline solution of such digested pigment, as in the previous experiment, the acid hæmatin remains indefinitely in suspension.

Experiment (4).

A solution of pure hæmatin, prepared from hæmin crystals, was made in N/10 NaOH. It was found that the acid hæmatin precipitated immediately when this was acidified. The rate of sedimentation was even more rapid than in the case of papain-extracted pigment. On the other hand, if the solution contained a little dissolved gelatine, then no flocculation or precipitation occurred on the addition of HCl.

The results of these experiments strongly support the view that, when a sufficient amount of protein is present in an alkaline solution of hæmozoin or of hæmatin, the addition of acid produces the formation of acid hæmatin which is held in colloidal suspension. To obtain additional evidence in support of these findings, some dialysis experiments were made.

Experiment (5).

An alkaline solution of hæmozoin derived from fresh parasites was made in N/10 NaOH. This solution was dialysed for 30 hours against distilled water, as in experiment (1), and similar results were obtained. This proved that practically all the pigment in the alkaline solution was in a colloidal state, and only a very small fraction in true solution. If this be so, then it appears safe to infer that the hæmozoin in aqueous solutions of HCl is also in a colloidal condition.

When solutions of pure hæmatin derived from hæmin crystals were made in N/10 NaOH or in N/10 KOH, the results obtained on dialysis were similar to those obtained with malarial pigment solutions.

The results support the view that the action of HCl on the pigment in alkaline solutions of malarial parasites is one of peptisation due to the presence of proteins. The latter were derived from the remains of the parasites in the original pigment suspension.

Brown (1912) reports that he obtained two kinds of alkaline hæmatin solution, one clear and one turbid. He considered that the latter was of a colloidal nature. Keilin (1926) has recorded that when acid is added to defibrinated blood, the acid hæmatin so formed is kept in suspension by the globin and other proteins present in the blood. These observations appear to be analogous to our results.

(2) Ammonium sulphide.

The pigment, both from parasites and from fresh splenic tissue, was found to be easily soluble in ammonium sulphide. This confirms the results of other workers who used fixed tissues.

(3) Mineral acids.

One of the earliest properties of hæmozoin to be recognised, was its insolubility in concentrated HCl or H_2SO_4 . This character served to differentiate it from hæmosiderin. We have found that H_2SO_4 does not dissolve but decomposes hæmozoin, as shown by the formation of hæmatoporphyrin detectable spectroscopically. Hæmozoin was found to be insoluble in HNO_3 as reported by Warasi (1927). Glasunow (1925), however, states that it is decomposed by this acid. Our observations confirm those of the latter worker. The oxidising action of HNO_3 was apparently so very rapid that it was impossible to demonstrate the presence of hæmatoporphyrin, as in the case of decomposition with H_2SO_4 . Hæmozoin is insoluble in concentrated HCl.

In spite of the insolubility of malarial pigment in concentrated or aqueous solutions of the mineral acids, it is easily soluble in acid solutions of alcohol, acetone, ether or chloroform. This action of acid alcohol has been recognised by Brown (1911), Stevenson (1917), Hueck (1921), Mayer (1923), Seyfarth (1926) and many other observers.

The action produced by the addition of strong acids to alkaline solutions of hæmozoin and hæmatin has been discussed in detail under the solvent action of alkalis.

(4) Organic acids.

Mayer (1923) and Warasi (1927) report that malarial pigment is slightly soluble in acetic acid. Glasunow (1925) states that it is soluble very quickly in glacial acetic acid with heat. On the other hand, Wats and White (1932) regard it as insoluble in 5 per cent alcoholic solution of this acid.

We have found malarial pigment to be soluble in glacial acetic acid in the cold (room temperature) in about 6-7 hours, but the rate of solution is markedly accelerated by heating. In the latter case only about 1 hour was necessary.

The insolubility of hæmozoin in oxalic acid, as recorded by Brown (1911), Basu (1921), Glasunow (1925) and other workers, has been confirmed. Solutions could be obtained with formic and butyric acids as quickly as with acetic acid.

(5) Miscellaneous reagents.

Hæmozoin was found to be insoluble in chloroform, acetic ether, petroleum ether, ethyl alcohol, methyl alcohol, amyl alcohol and carbon bisulphide. These findings are in agreement with those of many other workers.

Welch (1897) reports that it has been found to disappear under the action of calcium chloride. We have been unable to confirm this when pure reagents were used.

Glasunow (1925) states that malarial pigment is very slightly soluble in aniline, in pyridine and in 4 per cent solution of quinine in chloroform. He found that complete solution may take as long as 14 days depending on the size of the granules. Our findings were that hæmozoin granules were completely dissolved in about 40 hours in all these solutions, while hæmatin dissolved in about 1 hour.

(6) Discussion.

The comparative solubility tests which were carried out at the same time with pure hæmatin have been contrasted in Table A. From this table it will be seen that the reactions to the different solvents appear identical, except that the rate of action with pure hæmatin appears to be more rapid than with the pigment in the parasite suspensions.

From our observations upon the action of different reagents, both mixed and pure, a very close resemblance has emerged between the solubilities of hæmozoin and of hæmatin. It has been found, under the conditions of our experiments, that these two pigments are soluble in the same reagents, while those substances which have no solvent action on one pigment have none on the other. Such a result cannot be interpreted as merely a chance coincidence in so many experiments. The coincidence shows that there is a very close resemblance between, and we believe an actual identity of, parasitic hæmozoin and hæmatin. It remains to be determined with which of the so-called different types of hæmatin malarial pigment is identical.

(b) DIFFERENCES IN THE RATES OF SOLUTION OF HÆMOZOIN AND HÆMATIN IN DIFFERENT SOLVENTS.

An attempt has been made in Table A to indicate the comparative rates of solution of these two pigments. These observations are, however, only of a semi-quantitative nature. The experiments were carried out in test tubes using about 3 c.c. of the solvents and 4 drops of a thick suspension of malarial parasites or hæmatin.

In the case of malarial pigment, the remains of the parasites present with the suspension of this substance often prevented an accurate determination of the time when complete solution had occurred. It may be roughly stated, however, that, under the conditions of our experiments, the rate of solution of parasitic hæmozoin appeared to be slower than that of hæmatin with all the solvents used. Warasi (1927), in his table of solubilities, also notes that the rate of solution of malarial pigment in acid ether and in acetic acid, is slower than that of hæmatin. It might, therefore, be argued that, if the two substances be identical, such a difference should not be present.

The solubility of a solute is its maximum weight which a fixed weight (usually 100 gms.) of a solvent can dissolve at a fixed temperature. It is this

feature which is a more characteristic property of a compound than is its rate of solution. Even with the same solute and the same solvent at the same temperature, the rate of solution is influenced by a number of factors of a purely physical nature. Of these the following seem specially related to our problem.

(i) The first condition essential for solution is that the solute and the solvent should be in intimate contact with each other. If a crystal of NaCl be coated with a thin layer of paraffin wax it will not dissolve until the coating is removed or injured, so as to allow some access of the solvent.

(ii) The rate of solution for the same mass of solute and solvent depends upon the area of the solid-liquid interface. Thus a large crystal of a substance like CuSO_4 will dissolve less rapidly than will the same mass in a finely powdered form. The powdering causes a great increase in the possible solid-liquid interface.

(iii) The rate of solution also depends upon the rate of diffusion of the dissolved solute from the solid-liquid interface to the interior of the solvent. If the rate of diffusion be hindered by some factor, for example a semi-permeable membrane or a coating of another less soluble substance round the solid, then the solvent layer at the interface will soon be saturated and will not dissolve any more solute.

(iv) The rate of solution is influenced by the difference of concentrations of the solute in the interfacial solvent layer and that in the bulk of the solvent. It is a common experience that the rate of solution of a solute in a solvent which is nearly saturated with respect of the solute is very slow. This is due to the fact that the difference of concentration of the solute in the interfacial solvent layer and that in the bulk of the solvent is nearly zero.

On microscopical examination it was found that the granules of hæmozoin are coarser than those of the hæmatin suspension used by us. This factor alone would make the rate of solution of the latter substance more rapid than that of the former.

The hæmozoin used in our experiments was still enclosed in the remains of the parasites. This protein coating may retard the rate of solution in three different ways:—(i) by covering a portion of the surface of the pigment granules and thus diminishing the available solid-liquid interface, (ii) by retarding the rapidity with which the solvent can gain access to the solute, and (iii) by retarding the rate of diffusion of the dissolved solute from the interfacial layer to the interior of the solvent.*

On the other hand, the hæmatin used in our experiments was not covered by any such coating of protein to retard its rate of solution. If these factors be taken into consideration, it does not seem so surprising that the rate of solution of the malarial pigment studied by us, was in general slower than that of the hæmatin used.

It was also noted in the course of the investigation that the difference in the approximate rates of solution of the two pigments was less in the case of

*That the protein covering hinders the diffusion of the solution, is supported by the observation made by Mayer (1923) that the colour reaction produced by the iron of the pigment, is confined by the limits of the cell containing the pigment.

those solvents which had a destructive or solvent action on protein. Such an action would tend to remove the protective coating of protein around the granules of hæmozoin, and thus reduce the differences in the rate of solution of the two substances.

If the data discussed above be taken into consideration, the differences reported between the rates of solution of hæmozoin and of hæmatin are more easily explicable. These differences do not seem so important as some workers appear to believe, nor do they seem sufficient to negative the view that the two pigments are identical.

(c) THE POSSIBILITY OF CHANGES IN THE COMPOSITION OF THE PIGMENT
DUE TO THE ACTION OF SOLVENTS.

Much of the previous work recorded here and that of previous workers has been done with pigment extracted from the parasites by the use of alkaline solutions such as N/20 NaOH. It might possibly be argued that the original pigment in the parasites had been altered by the action of the solvent and that the substance studied by us was not true malarial pigment.

If hæmozoin be an unstable compound of some hæmatogenous pigment with a protein, then treatment with alkalis is likely to alter it. To study the true chemical nature of the pigment, it was necessary, therefore, to extract it by some method which was known to produce no change in its composition.

As the pigment used by us was found inside the mature parasites, it is evident that the latter cannot decompose it further. On the other hand, it has generally been accepted as proven that this pigment is formed, mainly at least, from the decomposition of the hæmoglobin of the red cells by the parasite. Under such conditions the pigment must be a more stable compound than hæmoglobin. Therefore, if it be possible to extract the pigment under conditions in which hæmoglobin is not decomposed, it appears reasonable to assume that the pigment has been extracted in an unchanged state.

A number of reagents, such as 0.04 per cent KOH in alcohol and a concentrated aqueous solution of NaHCO_3 , were tried with unsatisfactory results. It was found, however, that N/2 aqueous solution of Na_2CO_3 would dissolve out the pigment from the parasites, without decomposing the hæmoglobin.

Experiment (6).

An equal volume of N/1 Na_2CO_3 solution was added to 1.5 c.c. of a somewhat thick suspension of parasites in a narrow test tube. As a control experiment, 1.5 c.c. defibrinated blood, previously diluted with 3 parts of distilled water, was placed in a similar tube and mixed with an equal volume of the alkali solution. The tubes were left at room temperature for 2 hours, during which time they were gently shaken several times. They were then transferred to an incubator at 30°C . for 2 hours. After this period the solutions in the two tubes were examined spectroscopically. The hæmoglobin solution in the control tube showed the bands of oxyhæmoglobin only, while the hæmozoin solution showed the bands of alkaline hæmatin.

Two other experiments were carried out to confirm the above results. The fresh pigmented spleen from a malarial monkey was powdered in a glass mortar and freed from hæmoglobin by frequent washing with distilled water followed by centrifugalisation. As a control the spleen from a normal monkey was treated in a similar manner.

Experiment (7).

Approximately equal portions of the normal and the malarial spleens were treated in separate sample tubes with an equal volume of 0.04 per cent alcoholic solution of KOH. Hæmatin bands were detected in the solution from the malarial spleen and not in that from the normal spleen.

Experiment (8).

About the same weights of normal and malarial spleens were treated in the same manner with N/2 aqueous solution of Na_2CO_3 . As a further control the blood of a normal monkey, diluted 1 in 4 with distilled water, was mixed with an equal volume of the same alkaline solution. The blood solution showed on spectroscopical examination the bands of oxy-hæmoglobin, the solution from the normal spleen showed no absorption bands, while the solution from the malaria spleen showed those of alkaline hæmatin.

These experiments indicate that it is very improbable that the pigment in our solutions had been decomposed into hæmatin during the process of extraction. The absence of such decomposition is also supported by the fact that the numerous other solvents used produced solutions similar to those of hæmatin under like conditions (*vide* later section on spectroscopical examination). It is highly improbable that the same result could be obtained under such varied conditions, and that a solution of hæmatin would be produced, irrespective of the solvent used.

The results of these experiments support the views that (i) the pigment elaborated by the malarial parasite during its growth in the blood is probably identical with hæmatin and that (ii) the pigment extracted from malarial parasites and tissues is not produced from other hæmatogenous pigments by the action of the solvents used in extraction.

(d) SUMMARY.

(i) The actions of a large number of different solvents upon hæmozoin have been investigated, and the results compared with those observed with hæmatin. A very close resemblance in their reaction to solvents has been found between the two pigments.

(ii) The probable reasons for the recorded differences between the rates of solution of the two pigments have been investigated, and the possible causes of these discussed.

(iii) No evidence was obtained that the pigment extracted from malarial parasites or tissues had changed in composition due to the action of the solvents used for extraction of the substance.

TABLE A.

Action of different solvents on haemozoin compared with haematin.

	Solvent.	Hæmozoin.	Hæmatin.	REMARKS.
1	N/20 NaOH in aqueous solution ..	++	+++	These solutions are in a colloidal state (<i>vide</i> text).
2	N/20 KOH in aqueous solution ..	++	+++	
3	N/10 NH ₄ OH in aqueous solution ..	++	+++	
4	N/2 Na ₂ CO ₃ in aqueous solution ..	+	++	
5	0.04 per cent KOH in alcoholic solution	++	+++	
6	50 per cent (NH ₄) ₂ S in aqueous solution	++	+++	
7	Lithium carbonate, saturated aqueous solution.	+	++	
8	Calcium chloride	—	
9	5 per cent Na ₂ S in aqueous solution	++	+++	
10	Acetic acid, glacial	+	++	The solution is accelerated by heat, with both pigments.
11	Formic acid	+	++	
12	Oxalic acid	—	—	
13	Butyric acid	+	++	
14	HCl, concentrated	—	—	
15	H ₂ SO ₄ , concentrated	—	—	Decomposed in cold.
16	HNO ₃ , concentrated	—	—	Decomposed in cold.
17	Solutions of HCl, H ₂ SO ₄ , or HNO ₃ :—			
	(a) Aqueous (2 per cent) ..	—	—	
	(b) Alcoholic (6 per cent) ..	+	++	
	(c) Acetone (5 per cent) ..	+	++	
	(d) Chloroform (3 drops acid in 3 c.c. solvent).	+	++	
	(e) Ether (3 drops acid in 3 c.c. solvent).	+	++	
18	Pyridine	+	++	
19	Aniline †	+	++	

* The comparative rates of solution are indicated approximately in the table. A rapid rate of solution is indicated by + + +, a less rapid one by + +, and a slow rate by +, while — indicates that no solubility could be demonstrated.

† The aniline was purified by steam distillation and then dehydrated with K₂CO₃.

TABLE A—concl'd.

	Solvent.	Hæmozoin.	Hæmatin.	REMARKS.
20	Chloroform	—	—	
21	Ether, acetic	—	—	
22	Ether, petroleum	—	—	
23	Quinine, 4 per cent solution in chloroform.	+	++	
24	Alcohol, ethyl	—	—	
25	Alcohol, methyl	—	—	
26	Alcohol, amyllic	—	—	
27	Carbon bisulphide	—	—	

V. SPECTROSCOPICAL EXAMINATION OF SOLUTIONS OF HÆMOZOIN.*

Carbone (1891) appears to have been the first observer to study malarial pigment by spectroscopical methods and to note its resemblance to hæmatin. Ascoli (1910, 1915) obtained the pigment by the splenic puncture of cases of pernicious malaria, and from its spectroscopical appearance also suggested that it was hæmatin.

The first exhaustive study of hæmozoin by this method was that of Brown (1911), who reported that alkaline solutions of malarial pigment showed the bands of alkaline hæmatin, while acid solutions showed those of acid hæmatin. The reduction caused by the addition of Stokes' reagent to a hæmozoin solution, produced the bands of hæmochromogen and the addition of strong mineral acid those of acid hæmatoporphyrin. These results are similar to those obtained with solutions of hæmatin treated in the same manner.

Seyfarth (1921) confirmed the results of Carbone and of Brown. Glasunow (1925) treated malarial pigment with oxalic acid for 24–28 hours to remove any hæmosiderin which might be present. He found that such pigment gave the usual spectrum of hæmatin when dissolved in 0.04 per cent alcoholic solution of KOH. Solutions of hæmozoin treated with $(\text{NH}_4)_2\text{S}$ at first showed the bands of hæmochromogen alone, but on standing for some time the spectra were those of a mixture of this pigment with hæmatin.

Rocchi (1931) extracted hæmozoin from malarial tissue by the complicated method devised by Warasi (1927), and records that he observed the bands of hæmatin in a solution made with a saturated aqueous solution of lithium carbonate. On the other hand, Warasi (1927), by the same method of extraction, states that from a spectroscopical examination of such pigment he was unable to obtain any characteristic picture.

Basu (1921) extracted fresh malarial liver with a 1 per cent solution of HCl in acetone, but found that the spectroscopical appearances were obscured by the bile pigments present in the solution. However, in a later experiment in which he used portions of liver which had been preserved in Kaiserling's

solution, he observed the bands of hæmatin. Wats and White (1932) failed to demonstrate the spectrum of hæmatin in alkaline extracts of malarial blood or spleen.

These observations show that the majority of workers, who made spectroscopical examinations of solutions of malarial pigment, were able to demonstrate the presence of hæmatin in such solutions. As most of these experiments were done with fixed tissue pigment, it is necessary to confirm these results by an examination of fresh parasitic pigment. The possible fallacies in the former work have already been discussed.

Our examinations were made with a Zeiss direct vision spectroscope fitted with a wave-length scale.* The results were controlled by comparison with the appearances given by similar solutions of pure hæmatin prepared from hæmin crystals, and with the recognised properties of hæmatin and its derivatives, as reported by other workers.

A comparison between the results obtained with hæmozoin and with hæmatin under the same experimental conditions has been given in Table B.

(a) EXPERIMENTS WITH FRESH PARASITIC PIGMENT.

(1) Spectra of alkaline solutions.

The pigmented-parasite suspension was treated with N/20 aqueous solution of KOH. The tube was shaken at intervals for a short time, and then centrifuged at the end of 2 hours. The resultant pigment solution was carefully decanted and used for spectroscopical examination.

(i) *Alkaline hæmatin.*

In the alkaline solution of hæmozoin, a main band in the yellow-red extending from $625 \mu\mu$ to $595 \mu\mu$ was distinctly visible. There was also a very feeble absorption extending up to $575 \mu\mu$, as well as a general darkening stretching from the green to the violet end of the spectrum.

This appearance corresponds with the characteristic spectrum of alkaline hæmatin. As with the latter substance, the main band in the yellow-red spectrum became better defined on the addition of an equal volume of absolute alcohol.

(ii) *Alkaline hæmochromogen.*

It is a well-recognised property of hæmatin solutions that, when reduced in alkaline solutions, this pigment can combine with denaturised proteins to form hæmochromogen. The latter pigment shows two absorption bands in the green, one prominent and the other faint.

The alkaline solutions used in our work contained not only pigment, but also proteins extracted from the remains of the protoplasm of the parasites. Hence, if the pigment in the solution be hæmatin, it should show the spectral appearances of hæmochromogen on reduction.

On treating an alkaline solution of hæmozoin with Na_2S and adding some alcohol, the two absorption bands of hæmochromogen were observed in the green, with their centres about $558 \mu\mu$ and $527 \mu\mu$ respectively.

* The wave lengths (λ) of the spectral bands have been expressed in this paper as $\mu\mu$ i.e., 10^{-7} cm.

(iii) *Discussion.*

The observation that the absorption bands of alkaline hæmatin are seen in solutions of malarial pigment confirms that of several other workers.

It was found that when such solutions were diluted more and more, the absorption bands became fainter and fainter, and, when a certain dilution was reached, were no longer detectable. Wats and White (1932) failed to find such bands in the pigment solutions examined by them. It appears possible, therefore, that the solutions used by these workers were either too dilute to give a satisfactory result, or that they did not contain hæmozoin.

(2) *Spectra of acid solutions.*(i) *Acid hæmatoporphyrin.*

As noted in the section on solvents, the addition of concentrated H_2SO_4 to alkaline solutions of hæmatin decomposes the pigment, and the bands of hæmatoporphyrin are obtained.

(ii) *Acid hæmatin.*

Two c.c. of glacial acetic acid were mixed with about 3 c.c. of the original alkaline solution to keep the pigment in solution, and then a drop of strong HCl was also added to form acid hæmatin, according to the method recommended by Cole (1920).

On spectroscopical examination bands corresponding to those of acid hæmatin were seen.

A layer of ether about 4 cm. deep was then poured on the top of this acid solution. The tube was gently shaken and the ethereal layer examined. A prominent band was seen in the red with its centre about $638 \mu\mu$. In addition two other bands were observed, one in the green between $555 \mu\mu$ and $530 \mu\mu$, and the other in the blue-green between $515 \mu\mu$ and $495 \mu\mu$. The latter band was very faint. These appearances correspond with those of acid hæmatin in ethereal solution.

Acid solutions of malarial pigment in alcohol, acetone, ether and chloroform showed the absorption bands of acid hæmatin. The position of these bands varied slightly according to the nature of the solvent used. Gardner and Buckmaster (1913) record a fourth band in alcoholic and acetone solutions of acid hæmatin. This band is faint near the D line with a maximum at $578 \mu\mu$. We were unable to detect this band definitely in the dilute solutions used by us.

Solutions in acetic acid show a prominent band between $655 \mu\mu$ and $630 \mu\mu$, and another in the green between $557 \mu\mu$ and $535 \mu\mu$. The spectra of butyric acid solutions are similar, but those with formic acid are fainter.

(3) *Spectra in ammonium sulphide solutions.*

It was found that, if a small quantity of $(\text{NH}_4)_2\text{S}$ (about 0.5 c.c.) insufficient for solution be added to about 0.3 c.c. of a thick suspension of hæmatin, hæmin or hæmozoin respectively, a coarse suspension of these pigments is formed. If after about $\frac{1}{2}$ hour more $(\text{NH}_4)_2\text{S}$ be added, a somewhat turbid solution is obtained, which gives two absorption bands in the green. The band

between $578\ \mu\mu$ and $568\ \mu\mu$ is very prominent and the other band between $545\ \mu\mu$ and $530\ \mu\mu$ is faint. On standing at room temperature (about $20^{\circ}\text{C}.$) for 3 or 4 hours, the bands did not change. If alcohol be now added to this suspension, its turbidity diminishes. On spectroscopical examination the two characteristic bands of ammonia-haemochromogen appear and the two original bands fade away.

Anson and Mirsky (1925) observed the two original bands, described above, when they added a very small amount of ammonia or pyridine to a reduced haematin solution. On adding more and more ammonia, in small amounts at a time, they noticed a shift of these bands towards the violet end of the spectrum until the usual haemochromogen bands between $562\ \mu\mu$ and $548\ \mu\mu$ and between $535\ \mu\mu$ and $515\ \mu\mu$ were reached. Further addition of ammonia produced no further movement of the position of these bands. These authors concluded, therefore, that reduced haematin can combine with ammonia in different proportions. The compound formed by the addition of a very small amount of ammonia they termed α haemochromogen, and that formed by an excess of that reagent they called β haemochromogen.

Keilin (1926) found that, on adding salts like NaCl , NH_4Cl , etc., to a solution of β haemochromogen, the bands of α haemochromogen can be made to appear. He thinks, therefore, that the so-called α and β haemochromogens are one and the same compound, and that they differ only in the degree of dispersion of the particles of the pigment.

We have found that starting with a dilute solution (suitable for spectroscopical examination) of the so-called α haemochromogen in $(\text{NH}_4)_2\text{S}$, the β haemochromogen bands can be exclusively obtained after 15–20 minutes by adding alcohol only to the suspension. The addition of alcohol causes a diminution in the turbidity of the suspension and hence increases the dispersion of the haemochromogen particles. As the addition of alcohol alone cannot bring about any increase in the ammonia content of the so-called α haemochromogen, therefore our results go to confirm the view expressed by Keilin (1926) that these two haemochromogens are the same compound.

(4) Spectra in sodium sulphide solutions.

It has been found that when haemozoin is dissolved in a 5 per cent aqueous solution of Na_2S , the bands of alkaline haematin and the band of α haemochromogen appear. At first the α band is very faint, but after 15–20 minutes it becomes much more distinct. When haematin is treated similarly, the alkaline haematin bands are prominent, while the α haemochromogen band is extremely faint and does not become more intense on standing. This difference requires explanation.

It must be remembered in this connection that haemochromogen is only formed when reduced haematin can combine with protein or other nitrogenous compounds. The difference in behaviour between haematin and haemozoin was almost certainly due to the fact that the haematin was nearly pure and so free from protein, whereas the malarial pigment suspension contained sufficient amounts of protein because it was associated with the remains of parasite protoplasm. On the addition of 2 drops of egg-albumen, or 0.1 c.c. of ammonia solution, to 3 c.c. of the haematin solution, the haemochromogen bands appeared very soon, as in the case of haemozoin.

(5) Spectra in pyridine solutions.

It will be seen from a reference to Table B, that with pyridine, hæmatin first forms the compound pyridine-parahæmatin. This substance shows two absorption bands, one between 577μ and 575μ , the other between 545μ and 515μ . The position of these bands was first observed by Anson and Mirsky (1925).

We have found that if this compound be left with an excess of pyridine, it is completely transformed into pyridine-hæmochromogen in about an hour's time. It was, at first, thought that this reduction was possibly brought about by some reducing agent, present as an impurity in the sample of pyridine used. However, on the addition of potassium permanganate to the pyridine reagent, the pink colour did not disappear even in 24 hours, which does not support the suggestion that the pyridine contained an impurity with reducing properties. We were, therefore, inclined to believe that pyridine itself can bring about the reduction of the pyridine-parahæmatin compound. In a later experiment, the pyridine was distilled after mixing with a few drops of N/10 KMnO_4 . The pyridine thus purified showed parahæmatin bands only with both hæmozoin and hæmatin. No further reduction of pyridine-parahæmatin to pyridine-hæmochromogen could be observed. This experiment indicates that the pyridine originally used, although labelled 'extra pure', must have contained a trace of reducing substance, which was removed only after boiling with KMnO_4 .

With malarial pigment and pyridine*, the β hæmochromogen band was observed after about an hour, but the intermediate parahæmatin bands could not be detected. The reason for this is probably the fact that malarial pigment dissolves more slowly than does hæmatin (*vide* section on solvents). Because the former pigment dissolves slowly, the small amount of pyridine-parahæmatin produced is quickly transformed to pyridine-hæmochromogen, i.e., the pyridine-parahæmatin is never present in sufficient concentration to be detectable by its absorption spectra.

(6) Spectra in aniline solutions.

In aniline solutions, both hæmatin and hæmozoin form a compound which shows two absorption bands in the green. So far as we know this fact has not been recorded previously. Within the limits of experimental error, these bands occupy the same positions as do those of pyridine-parahæmatin. The name aniline-parahæmatin is, therefore, proposed for this compound of aniline and hæmatin. No further change to hæmochromogen was observed in aniline solutions.

(7) Summary.

(i) The spectra of hæmozoin in different solutions have been observed, and the action of various reagents on such spectra studied.

(ii) The results have been compared with those obtained with hæmatin under similar conditions, and have been found to be identical.

(iii) The probable identity of α and β hæmochromogens has been investigated, and a new compound, aniline-parahæmatin, recorded.

(b) EXPERIMENTS WITH TISSUE PIGMENT.

In addition to the observations made upon the pigment from parasites, some experiments were also carried out with tissue pigment both fresh and fixed.

* Impure.

(1) Fresh tissue.

Pieces of fresh malarial spleen from cases of acute monkey malaria were ground to a pulp in a glass mortar, and then freed from hæmoglobin by repeated washings with distilled water and centrifugalisation. When alkaline solutions were made of the residue, the characteristic spectra of alkaline hæmatin were obtained. Controls of spleens and blood clots from normal monkeys treated in the same way showed no such absorption spectra.

(2) Fixed tissue.

Some investigations were made on small pieces of splenic tissue from acute cases of monkey malaria. These tissues were fixed in Zenker's solution and afterwards carefully washed, dried in a desiccator and powdered. Approximately 0.5 gm. of the powder was treated in a test tube with 10 c.c. of 0.13 per cent alcoholic solution of NaOH. The tube was shaken several times at intervals of 15–20 minutes and then left undisturbed for 2 hours. The supernatant fluid showed the bands of alkaline hæmatin.

This technique is similar to that used by Brown (1911), except that soda was used instead of potash. Brown states that by this method, although hæmozoin is extracted, hæmoglobin is not. To control this work the following experiments were carried out:—(1) a clot of blood from a normal monkey was fixed and extracted as described above, and (2) a piece of spleen from a normal monkey was treated in the same manner. Extracts made with 0.04 per cent KOH in alcohol showed no signs of hæmatin in either instance.

TABLE B.

*Results of spectroscopical examination of solutions of hæmozoin compared with hæmatin.**

	Solvents.	Hæmozoin.	Hæmatin.
1	Aqueous solutions of (a) N/20 NaOH, (b) N/20 KOH, (c) N/10 NH ₄ OH, (d) N/2 Na ₂ CO ₃ , (e) Li ₂ CO ₃ , saturated.	Spectrum of alkaline hæmatin. A prominent band between = 625 $\mu\mu$ and 595 $\mu\mu$. A feeble absorption extending up to 575 $\mu\mu$. Blue end of spectrum absorbed.	As with hæmozoin.
2	0.04 per cent KOH in alcoholic solution.	Spectrum of alkaline hæmatin as described above. The band in the yellow-red is, however, more definite.	As with hæmozoin
3	50 per cent (NH ₄) ₂ S in aqueous solution.	The two bands of hæmo-chromogen seen in green. The prominent α band shows maximum light absorption about 557 $\mu\mu$ and the feeble β band has its centre about 527 $\mu\mu$.	As for hæmozoin.

* The wave lengths (λ) have been expressed in this paper as $\mu\mu$, i.e., 10^{-7} cm.

TABLE B—concl'd.

	Solvents.	Hæmozoin.	Hæmatin.
4	5 per cent Na_2S in aqueous solution.	At first alkaline hæmatin bands and faint hæmochromogen bands. On standing the latter become marked and the former fade.	Prominent alkaline hæmatin spectrum. Hæmochromogen α band extremely faint, β band not visible. Latter bands become prominent after addition of ammonia or egg-albumen (<i>vide text</i>).
5	Acetic acid, glacial ..	A prominent band between $655 \mu\mu$ and $630 \mu\mu$. Another in green between $557 \mu\mu$ and $535 \mu\mu$.	As for hæmozoin.
6	Formic acid ..	As for acetic acid but fainter.	As for hæmozoin.
7	Butyric acid ..	As for acetic acid.	As for hæmozoin.
8	H_2SO_4 , concentrated ..	Bands of acid hæmatoporphyrin.	As for hæmozoin.
9	6 per cent alcoholic solutions of HCl , H_2SO_4 , or HNO_3 .	A prominent band in red with maximum absorption about $632 \mu\mu$. Two other bands in green—one between $555 \mu\mu$ and $535 \mu\mu$, the other near the F line.	As for hæmozoin.
10	5 per cent acetone solutions of HCl , H_2SO_4 , and HNO_3 .	A prominent band in the red with maximum absorption about $630 \mu\mu$. Two other bands visible in green.	As for hæmozoin.
11	Chloroform, 3 c.c. plus 3 drops HCl , H_2SO_4 , or HNO_3 .	Prominent band in red between $655 \mu\mu$ and $630 \mu\mu$. Two other bands—one between $557 \mu\mu$ and $535 \mu\mu$, and one in blue-green.	As for hæmozoin.
12	Ether, 3 c.c. plus 3 drops of HCl , H_2SO_4 , or HNO_3 .	Prominent band in red between $650 \mu\mu$ and $630 \mu\mu$. Two other bands in green, one between $555 \mu\mu$ and $530 \mu\mu$, the other between $512 \mu\mu$ and $498 \mu\mu$.	As for hæmozoin.
13	Pyridine	Two pyridine-hæmochromogen bands in green. The feeble β band appears after about an hour.	At first 2 bands appear in green between $575 \mu\mu$ and $557 \mu\mu$ and between $515 \mu\mu$ and $545 \mu\mu$, but after an hour they are replaced by pyridine-hæmochromogen bands (<i>vide text</i>).
14	Aniline	Two bands in green between $575 \mu\mu$ and $557 \mu\mu$, and $545 \mu\mu$ and $515 \mu\mu$ (<i>vide text</i>).	As for hæmozoin.
15	Quinine (4 per cent) in chloroform.	The bands of alkaline hæmatin as described in (1).	As for hæmozoin.

VI. CONCLUSIONS.

It has been found, under the conditions of our experiments with hæmozoin derived from unfixed parasites, that (a) there is a very marked resemblance between the action of various solvents upon this pigment and upon hæmatin, and that (b) the spectroscopical appearances observed in solutions of these two pigments coincides very closely.

The results obtained confirm the view of many other workers that hæmozoin is very closely allied to hæmatin, but it is still uncertain with which so-called type of the latter pigment it is probably identical.*

We wish to express our thanks to Jemadar Harbhagwan, I.M.D., for much help given during the progress of these investigations.

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* Recent work suggests that there is only one type of hæmatin, not several as suggested by other workers (*vide* Pryde, 1928).

† It has not been possible to consult many of the older works on melanosis and melanemia in the original. The information given in this paper has, therefore, been derived mainly from Frerichs (1858), Laveran (1907), etc., who have summarised most of the earlier work. The subject of malarial pigment is dealt with in many of the textbooks on malaria. As most of the information recorded in such works appears to be derived mainly from the observations of other authors, it has not been considered necessary to include these in this list of references.

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STUDIES OF MALARIAL PIGMENT (HÆMOZOIN).

Part II.

THE REACTIONS OF HÆMOZOIN TO TESTS FOR IRON.

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I. INTRODUCTION.

THE investigations described in a previous paper (Sinton and Ghosh, 1934) showed that malarial pigment, extracted from the parasites of the circulating blood, resembles hæmatin both in its spectroscopical appearances and in its reactions to various solvents. As mentioned in that study it appears very probable that hæmozoin contains iron. Some workers have expressed considerable doubt as to (a) the possibility of demonstrating iron in such pigment by micro-chemical methods, and as to (b) whether the records of such successful demonstration might not be due either to changes in the pigment caused by fixation, or to the presence of undetected hæmosiderin along with it.

Perls (1867) reported that all the dark pigment granules seen in malarial tissues did not show a blue coloration with the Berlin-blue reaction. Marchiafava and Celli (1884) noted that not even the finest pigment granules in the red cells gave a positive reaction with this test. Neumann (1889) was unable to obtain the iron reaction with malarial pigment, and Stieda (1893) found that, while many pigment granules in malarial tissue gave the Berlin-blue reaction, those in the capillaries did not. Welch (1897) states that Neumann, Stieda, Dock and others were unable to demonstrate iron in malarial pigment. Dutton (1898), however, recorded that the use of the Berlin-blue reaction, after preliminary treatment of the sections with an alcoholic solution of HNO_3 , gave a positive result with the 'melanin' granules of malarial tissues.

While several of the more modern writers state that malarial pigment is iron-free, this is apparently not meant to indicate that the metal is absent from hæmozoin, but rather that it is not present in a form detectable by the usual chemical tests. As was pointed out by Craig (1909), the mere fact that iron has not been demonstrated by such methods does not prove that it is absent.

The micro-chemical tests used by the older observers were the Berlin-blue and Turnbull-blue reactions, and the negative results of such tests have long been recognised as important characteristics of malarial pigment. It is now known that these tests will only reveal the presence of iron, if it be in an ionisable or inorganic form. It is, therefore, evident that if there be iron in hæmozoin, it must be present in a non-ionisable, organic or 'masked' condition. If it be desired to reveal such iron by the Berlin-blue or Turnbull's reaction, it is necessary to 'unmask' it first, that is to convert it into an ionisable form, i.e., ferric or ferrous ions. Different workers have devised techniques to unmask non-ionisable iron in the tissues, and these methods have depended upon preliminary treatment with various oxidising or reducing agents.

Macallum (1896) reported that alcoholic solutions of H_2SO_4 and HNO_3 were superior to Bunge's fluid (an alcoholic solution of HCl) for this purpose. Tracy (1905) recommends preliminary treatment of the tissue with H_2SO_4 (4 per cent) in alcohol. Lee (1928) states that 4 per cent alcoholic solution of H_2SO_4 or HNO_3 is better than a similar solution of HCl . The H_2SO_4 acts very slowly and may take 24 hours to unmask organic iron, while the HNO_3 is quicker and extracts very little of the metal which it liberates.

Brown (1911a), after preliminary treatment with dilute solutions of hydrogen peroxide, was able to demonstrate the iron reaction with hæmin crystals and with hæmatin, using the Berlin-blue test. He also states that other oxidising reagents, such as potassium bichromate, chromic acid, potassium permanganate and potassium chlorate, may be used, but that chromium salts are liable to cause confusion in sections.

Macallum (1891) used an ammonium-sulphide glycerine mixture to unmask iron. Hueck (1912) reports that, after using $(\text{NH}_4)_2\text{S}$ to unmask non-ionisable iron, he was able to obtain a positive Turnbull-blue reaction.

Various modifications of these micro-chemical methods have been used by different workers in attempts to demonstrate the presence of iron in hæmozoin.

granules. Brown (1911), Seyfarth (1921, 1926) and Mayer (1923) claim successful results with acid-alcohol, followed by the Berlin-blue reaction. Basu (1921) and Glasunow (1925) failed to confirm these reports. Glasunow (1925) was also unsuccessful in demonstrating iron by the H_2O_2 method of Brown (1911a). Hueck (1912, 1921), Seyfarth (1921, 1926) and Mayer (1923) report a positive Turnbull reaction after the preliminary use of $(NH_4)_2S$, but Lignac (1923) and Glasunow (1925) could not confirm these results.

II. POSSIBLE FALLACIES IN THE METHODS USED TO DEMONSTRATE IRON IN THE MALARIAL PIGMENT OF TISSUES.

Although most workers do not deny that hæmozoin may contain iron, there is considerable diversity of opinion as to the validity of the methods used to demonstrate it.

(i) Lignac (1923) notes that prolonged treatment with HCl and $K_4Fe(CN)_6$ has usually been necessary to demonstrate the Berlin-blue reaction with the 'unmasked' iron of hæmozoin. He points out that if these reagents be kept in contact for a considerable period, even in the absence of extrinsic iron, a blue coloration may be produced. We have also found that such a coloration may develop in this mixture after about an hour. It is necessary, therefore, to obtain the Berlin-blue reaction in a more rapid manner, if the test is to be free from fallacies.

(ii) Lignac (1923) also considers that another possible fallacy is present in the results of Brown and Seyfarth. This is that these workers were only able to obtain a blue halo *around* the pigment granules and not *inside* them. Mayer (1923), however, reports the reaction inside the granules.

(iii) Most observers, who have recorded a positive iron reaction with malarial pigment, have worked with fixed tissues, and in several instances the fixatives used have contained formalin. It is well known that, by the action of formalin upon blood, a pigment is produced in tissues fixed with this reagent. The pigment produced shows very many of the characters of hæmozoin, and is also derived from hæmoglobin. To eliminate the possibility of such a fallacy it is necessary to work with unfixed material, if possible.

(iv) Even if it be admitted that, by the use of various unmasking methods, it is possible to demonstrate iron micro-chemically in the hæmozoin of tissues, it must be remembered that attempts to obtain similar reactions with the pigment of parasites have usually failed in the past. This suggests that some change takes place in the nature of the pigment after it has been phagocytosed. It seems quite reasonable to suppose that the phagocytes may have some action on ingested pigment. Such an action would probably be produced by intracellular enzymes, and Kósa (1925) reports that parasitic hæmozoin can be unmasked by the action of gastric juice. Under such circumstances, while the demonstration of iron in phagocytosed pigment would be very strong presumptive evidence that this metal was also present in parasite pigment, yet it would not prove that the iron was combined in the same form under these two conditions.

(v) As much of the tissue hæmozoin is contained in reticulo-endothelial cells, it must be remembered that such cells may also contain granules of hæmosiderin. The latter pigment may be present in large amounts in tissues, such as the liver and spleen, under conditions where excessive destruction of the

erythrocytes is occurring. An intense direct Berlin-blue reaction is, as a rule, given by this pigment. The simultaneous occurrence of these two pigments in malarial tissues has led to considerable confusion in the past.

Lubarsch (1925) has pointed out that, while Mayer (1923) records a positive reaction with pigment in macrophages, yet the latter worker failed to demonstrate it in the pigment of parasites still enclosed in adjacent red cells of the same section. Lubarsch (1925) suggests, therefore, that this difference would be explicable, if the positive iron reaction be due to the presence of undetected hæmosiderin in the same macrophages. A further investigation of parasite pigment is needed to exclude the possibility of such a fallacy.

(vi) It has also been suggested by some workers that the iron reactions obtained with the pigment granules in the tissues, may have been due to a coating of hæmosiderin or to an admixture of this substance with them. These suggestions require more detailed consideration.

(a) THE POSSIBLE OCCURRENCE OF HÆMOSIDERIN IN GRANULES OF HÆMOZOIN.

Hæmosiderin granules are deposited in the tissues during conditions of excessive destruction of red blood cells. This pigment occurs as yellow or orange granules, which appear brownish when seen *en masse*. This is the 'ochre pigment' of older workers, which was named hæmosiderin by Quincke.*

Many observers think that its formation is closely related to that of bile pigments, and that hæmoglobin is split into hæmosiderin and an iron-free residue from which the former pigments originate. The fact that there is a large destruction of red cells in malaria, means that the tissues will usually contain not only malarial pigment but in addition deposits of hæmosiderin and bile pigments. There has been considerable confusion between these hæmatogenous pigments in the past (*cf.* Ewing, 1903, 1905).

The most commonly recognised properties of hæmosiderin are that the Berlin-blue reaction is easily obtainable without any preliminary unmasking treatment, that it turns black or dark green under the action of $(\text{NH}_4)_2\text{S}$, and that it is insoluble in alkalis.† These reactions distinguish it from hæmozoin. It can be extracted from the tissues by aqueous solutions of acids, while alcoholic solutions of these reagents are needed to dissolve malarial pigment.

Neumann (1900) suggested that malarial pigment granules in the tissues are merely nuclei around which hæmosiderin is deposited. Brown (1911) envisaged the possibility that the iron reaction obtained by him with the pigment of tissue sections, might be due to the unsuspected presence of hæmosiderin. To exclude this possible fallacy in his work, he extracted his sections with either a 2 per cent aqueous solution of oxalic acid or with Pal's solution, as a preliminary measure. After such treatment, he unmasked the iron in his sections and obtained a positive Berlin-blue reaction with hæmozoin granules.

*Laveran (1907) states that hæmosiderin may be a variety of ferric hydrate, distinguished from ordinary ferric hydrate notably by its insolubility in cold acids. Adami (1909) suggested that it was hydrated ferrous oxide in combination with protein. Hueck (1921) states that it resembles sodium hydrated iron oxide in nearly all respects. Cook (1929) has shown that hæmosiderin is probably hydrated ferric oxide, and tentatively defines it as some form of colloidal ferric oxide. These workers are agreed that the iron in this pigment is present in an ionisable form, in contradistinction to that in hæmozoin.

† An intensive study of this pigment has been made by Cook (1929), to whose work the reader is referred for more detailed information.

Seyfarth (1921, 1926), Mayer (1923) and Kósa (1925) used a similar preliminary treatment with oxalic acid, and they all report that it was possible to demonstrate an iron reaction afterwards.

Hueck (1912) obtained blue rings around malarial pigment granules on several occasions, but thought that, while these might be due to the presence of hæmosiderin, yet the possibility of separation of iron from hæmozoin by his unmasking method could not be excluded.

Glasunow (1925) has studied this question in some detail. He believes that the positive results reported by Brown, Seyfarth and Mayer would only be possible (a) if the preliminary treatment with oxalic acid had failed to remove all the hæmosiderin, or (b) if either the acid-alcohol or the ammonium sulphide, used to unmask the iron, not only dissolved the pigment but also decomposed it (*vide* Section IV).

(b) EXTRACTION OF HÆMOSIDERIN WITH OXALIC ACID SOLUTIONS.

Glasunow (1925) notes that, while Mayer (1923) reports a positive reaction after sections of malarial tissue had been treated with 2 per cent solution of oxalic acid for 12 hours, yet he was not always successful when the acid had been allowed to act for about 18 hours. Glasunow thinks that these divergent results may be explicable by either of two factors:—(i) that the exposure of hæmozoin to oxalic acid for 16–18 hours may decompose the pigment and withdraw the iron, or (ii) that the hæmosiderin was incompletely extracted in the successful experiments reported by Brown, Seyfarth and Mayer.

The first suggestion is considered by Glasunow to be highly improbable, because he found that the pigment, even after 24–48 hours extraction with concentrated solutions of oxalic acid, still gave the spectrum of alkaline hæmatin, when dissolved in an alcoholic solution of KOH. In addition, the residue obtained after the evaporation of this solution was still negative to the ordinary tests for iron. Glasunow (1925) considers, therefore, that the incomplete extraction of hæmosiderin is a more likely explanation of the results. In our work we have been unable to obtain any evidence that hæmozoin is decomposed by aqueous solutions of oxalic acid.

Glasunow (1925) then investigated the suggestion that the oxalic-acid treatment used by other workers had failed to remove all trace of hæmosiderin from the tissue sections. He subjected his sections to 2 per cent solutions of oxalic acid for 24 hours or longer, and was then unable to demonstrate the presence of iron by any of the unmasking methods reported by other workers to give successful results. He concludes, therefore, that such results were dependent upon the incomplete extraction of hæmosiderin.

In our experiments steps were taken to eliminate the fallacies suggested by Glasunow (1925). The hæmozoin, and the hæmatin with which it was compared, were extracted with a 2 per cent aqueous solution of oxalic acid for 40 hours. They were then thoroughly washed with distilled water, before any attempt was made to unmask iron. As will be seen from the details of our experiments, we were still able to demonstrate the iron reaction with both pigments after this treatment.

Further experiments were undertaken to settle the question of the possible inclusion of hæmosiderin in the original pigment. We were unable to obtain a direct Berlin-blue reaction with fresh parasitic pigment, nor have we found

any blackening of this substance when treated with $(\text{NH}_4)_2\text{S}$. On the contrary this reagent had a solvent action (Sinton and Ghosh, 1934). Fresh malarial pigment was extracted with 10 per cent aqueous solution of HCl at 50°C . for $\frac{1}{2}$ hour. The resultant extract did not give the Berlin-blue reaction. With KCNS , a more delicate test for ferric iron, a very faint pink coloration was obtained. This result suggests that, if hæmosiderin or other ionisable forms of iron be present in hæmozoin, their amount must be very small. Macallum (1891) notes that acid-alcohol (HCl) is apt to extract some organic iron if allowed to act for a prolonged period at temperatures over 20°C . This may help to account for the faint reaction. It is also impossible to exclude the possibility that the faint reaction with KCNS may have been due to extrinsic iron introduced as dust, etc., in the process of isolating the parasites from the red cells.

The conclusions arrived at from these experiments were that hæmozoin granules, at least while inside the parasites, do not contain hæmosiderin in amounts sufficient to give any marked iron reaction with the usual micro-chemical methods used for detecting ionisable iron. This does not, however, exclude the possibility that hæmosiderin, or some other form of ionisable iron, may be produced from hæmozoin when this pigment is acted upon by the cells of the reticulo-endothelial system.

III. THE DEMONSTRATION OF IRON IN MALARIAL PIGMENT.

The attempts of previous workers to demonstrate iron in hæmozoin by micro-chemical methods have been discussed in some detail in earlier portions of this paper. It is evident from this discussion that there is no general agreement in the matter.

Most of the possible fallacies, which have been suggested by different workers, can be excluded, if the investigation be made with fresh malarial pigment derived from parasites only.

Our investigations have been carried out with such pigment. The methods used for the isolation of the pigment*, and for the preparation of the hæmatin used in comparative tests, have been described in a previous paper (Sinton and Ghosh, 1934). In all the techniques used in this research, the reagents employed were first tested to exclude the accidental presence of iron.

Although, as mentioned previously, no evidence could be obtained that parasitic malarial pigment was associated with hæmosiderin, both the hæmozoin and the hæmatin used in the tests were extracted with 2 per cent aqueous solution of oxalic acid for 40 hours, unless otherwise stated. Such a prolonged treatment was used as an additional safeguard against the possibility that any positive iron reaction obtained could be due to the previously undetected presence of hæmosiderin, or other forms of ionisable iron, although our preliminary tests had already given negative results.

Many workers have attempted to demonstrate the presence of iron in malarial pigment by micro-chemical methods, but few have used macro-chemical

* As in our previous work, the terms 'malarial pigment' and 'hæmozoin' have been used in this paper to denote a suspension of pigment still contained inside the remains of malarial parasites isolated from the circulating blood. This pigment is, therefore, under slightly different conditions from the hæmatin used for comparative purposes, as the latter had no such protein covering. This difference has already been discussed by Sinton and Ghosh (1934).

ones. Brown (1911) found, however, when a solution of hæmozoin in alkali was neutralised with HCl and evaporated to dryness, that the ash left after incineration contained iron. Warasi (1927) isolated from the tissues what he believed to be unchanged malarial pigment. An analysis was made of this substance, and the results are recorded as—carbon 51 per cent, hydrogen 7.6 per cent, nitrogen 13.5 per cent, oxygen 22.5 per cent and iron 2.9 per cent.

All our attempts to demonstrate ionisable iron in fresh malarial pigment derived from parasites have failed. These results, taken in conjunction with those of many other workers, confirm the view that if iron be present in hæmozoin it occurs in the 'masked', organic or non-ionisable form. Further investigations have been made, therefore, by both macro-chemical and micro-chemical methods to determine whether this element can be demonstrated in parasitic pigment by any of the various methods recommended to 'unmask' iron from its organic compounds.

EXPERIMENTS WITH VARIOUS UNMASKING METHODS.

Attempts have been made in these researches to reveal iron in the malarial pigment of unfixed parasites, by the unmasking methods with which other workers have recorded successful results. In several instances it has been found necessary to modify the techniques recommended.

A. HYDROGEN PEROXIDE.

Brown (1911a) reported that he was able to demonstrate the Berlin-blue reaction with granules of hæmatin and with crystals of hæmin, if these were first treated with H_2O_2 . Basu (1921) tried to unmask iron in malarial pigment by means of H_2O_2 and of KMnO_4 , but failed to obtain a positive reaction. Glasunow (1925) was also unable to obtain the Berlin-blue reaction, when he used Brown's method on sections of malarial tissue, nor could he obtain a positive reaction with KCNS after treatment of such sections with H_2O_2 . Glasunow, in addition, tried the action of this oxidising agent on the pigment mass obtained by neutralising and evaporating a solution of malarial pigment in alkaline alcohol. He removed the excess of H_2O_2 by taking advantage of the catalytic action of MnO_2 , even then he failed to reveal iron either by the Berlin-blue test or with KCNS.

We have, however, succeeded in demonstrating iron in parasitic hæmozoin, in hæmatin and in hæmin, when this element was previously unmasked by the action of H_2O_2 .

(i) Macro-chemical investigations.

Experiment (1).

The pigment was extracted from a thick parasite suspension by H_2O_2 , and the excess of this reagent removed from the resultant solution with MnO_2 . Like Glasunow (1925) we were unable to demonstrate iron in the extract.

It is now known, however, that cations are strongly adsorbed by MnO_2 (Ghosh, 1926), and this action appeared to be a possible explanation of our negative results and those of Glasunow (1925). To test this suggestion, the MnO_2 was separated from the solution and heated with HNO_3 . It was found that the acid extracted adsorbed iron from the MnO_2 , partially at least, and that the extract gave the reactions of ferric iron.

Experiment (2).

About 5 drops of a very thick suspension of malarial parasites in water were added to 4 c.c. of a strong solution of H_2O_2 .* When the pigment had become completely decolorised, the resultant solution was separated and evaporated to dryness in a porcelain dish over a water bath. This process completely removed any excess of H_2O_2 . The residue was then extracted by warming with 1 c.c. of distilled water and 1 drop of concentrated NH_4OH .

(a) When to one portion of this extract were added 2 c.c. of a strong aqueous solution of KCNS (about 35 per cent), the characteristic red reaction of ferric iron developed. Hæmatin treated in a similar fashion gave the same reaction.

(b) A second portion of the extract was treated with a few drops of a freshly prepared mixture, containing equal parts of 2 per cent aqueous solution of $\text{K}_4\text{Fe}(\text{CN})_6$ and 2 per cent aqueous solution of HCl . A positive Berlin-blue reaction revealed the presence of ferric iron. Hæmatin gave a similar result.

(ii) Micro-chemical investigations.*Experiment (3).*

Smears of malarial pigment, hæmatin and hæmin crystals were made on glass slides and allowed to dry at room temperature. A strong solution of H_2O_2 (12 vols. of O) was diluted 5 times with distilled water and the smears immersed in the fluid in closed jars. Slides were withdrawn at regular intervals, washed carefully with distilled water to remove the H_2O_2 completely. The smears were afterwards dried in the air, or by holding them at a height of about 9 inches above a non-luminous Bunsen flame. The smears were then treated with aqueous solutions of HCl and $\text{K}_4\text{Fe}(\text{CN})_6$, covered immediately, and examined under the microscope.

Result.—The hæmatin films gave the iron reaction when acted upon by H_2O_2 for 4 hours. Hæmin films showed the reaction after 6–8 hours, and the hæmozoin after 18–24 hours. As discussed in our previous article (Sinton and Ghosh, 1934), the delay with hæmozoin was probably explicable as due to the protein covering. This surrounded the pigment, and thus caused a slower penetration of the H_2O_2 than in the cases of hæmatin and hæmin.

Experiment (4).

A modification of the above method was found to be more convenient.

A drop of strong H_2O_2 solution was placed on the pigment films, and heated by holding the slide at a height of about 9–12 inches over a non-luminous Bunsen flame. When the drop of reagent was nearly evaporated, another drop was placed on the film and it was re-heated. The process was repeated 3 or 4 times. The film was then dried in the air, washed in distilled water, and tested for iron as in the previous experiment.

Result.—The blue ferric-iron reaction developed immediately with hæmozoin and hæmatin, showing that the reaction was not produced merely by the

* The strong solution of H_2O_2 used in our experiments was the 'Merckozone' preparation of Messrs. Merck, containing 12 volumes of O . It was acidified with a drop of HCl before use.

interaction of the reagents used, as was suggested by Lignac (1923). Hæmin crystals were not tested by this method.

(iii) Summary.

It has been found possible, by using H_2O_2 as an oxidising agent, to demonstrate the presence of iron in hæmozoin, hæmatin and hæmin, both by macro-chemical and micro-chemical methods. The reactions observed under the microscope occurred inside the granules themselves, and not merely in the form of haloes as reported by some other workers.

B. CONCENTRATED NITRIC ACID.

The action of concentrated HNO_3 and of H_2SO_4 on hæmozoin and on hæmatin results in the decomposition of these pigments (*vide* Sinton and Ghosh, 1934).

Experiment (5).

A small quantity of malarial pigment was added to about 3 c.c. of pure HNO_3 and, after acting for about 1 hour, the resultant extract was evaporated to dryness in a porcelain vessel over a water-bath. To the residue was added 1 c.c. of distilled water and 1 drop of concentrated HNO_3 , and the mixture warmed for a minute.

Result.—The extract was found to give the reactions of ferric iron with a strong aqueous solution of KCNS, and also with an aqueous solution of $\text{K}_4\text{Fe}(\text{CN})_6$ and HCl . Hæmatin treated in the same way gave a similar result.

Summary.

When hæmozoin and hæmatin granules were acted upon by pure HNO_3 for about an hour, it was possible to demonstrate the presence of ferric iron in the resultant decomposition products.

C. ALCOHOLIC SOLUTIONS OF MINERAL ACIDS.

The fact that hæmozoin is soluble in alcoholic solutions of mineral acids has long been recognised (*vide* Sinton and Ghosh, 1934). This property has been used by a number of workers in attempts to demonstrate the presence of iron in malarial pigment by micro-chemical methods.

Dutton (1898) reports that after preliminary treatment of sections of malarial liver or spleen with an alcoholic solution of HNO_3 (3 per cent), he was able to obtain the Berlin-blue reaction with malarial pigment. Brown (1911) treated sections of fixed malarial tissue with a mixture of acid-alcohol (2 per cent or stronger) and 2 per cent aqueous solution of $\text{K}_4\text{Fe}(\text{CN})_6$. The liquid was prevented from evaporating by the use of a coverslip, which was ringed with vaseline. He found that after some time a blue zone developed around the pigment granules. The reaction appeared more quickly if the preparations were warmed, or if the oxidising action of H_2O_2 were also used as a preliminary step. Seyfarth (1921) and Mayer (1923) also report successful results with acid-alcohol.

Basu (1921) and Glasunow (1925) tried this method, but failed to confirm the findings recorded by Brown. Warasi (1927) obtained an iron reaction with

malarial pigment by keeping a mixture of concentrated HCl and $K_4Fe(CN)_6$ in contact with the substance for 12–15 hours. He concluded that his sample of pigment was not hæmatin, because the latter gives this reaction more rapidly.

The successful results of this and other solvent methods seem to depend largely upon the duration of the action of the solvent. If the action be of very short duration, too little iron may be liberated to show a positive Berlin-blue or Turnbull reaction, while if too long a period be allowed, all the iron may leave the pigment granules and diffuse into the solvent.

Mayer (1923) considers that the negative results recorded by Basu (1921) with acid-alcohol and with $(NH_4)_2S$ were probably due to inattention to the time factor. Mayer (1923) also points out that no arbitrary time can be fixed for the preliminary action of the unmasking agent. The optimum time varies with each piece of tissue and can only be determined by trial. Seyfarth (1921) thinks that it is more difficult to demonstrate iron in tissues which have been subjected to prolonged fixation, but Mayer (1923) was unable to confirm this.

Glasunow (1925) extracted pigment from malarial tissue with 0.04 per cent KOH and subjected it to the action of acid-alcohol (HCl). He failed to demonstrate the Berlin-blue reaction in this solution.

(i) Macro-chemical investigations.

Experiment (6)

Our observations of dilute solutions of malarial pigment in 6 per cent solution of HNO_3 in alcohol, showed that while faint absorption bands of acid hæmatin were present at first, these could not be detected after 7 or 8 days. This suggests that there is a very slow decomposition of the pigment in alcoholic solutions of HNO_3 .

Result.—Tests by the Berlin-blue reaction for the presence of ferric iron in the solution were inconclusive, because the reddish colour of the extract masked the result. A distinct increase in the red colour was observed, however, when a strong solution of KCNS was added.

(ii) Micro-chemical investigations.

Experiment (7)

Dried films of hæmatin granules and of hæmin crystals were immersed in acid-alcohol (2 per cent HCl) for varying periods ($\frac{1}{2}$ –24 hours). They were then washed once with distilled water and finally treated with a mixture of acid-alcohol (2 per cent HCl) and 2 per cent aqueous solution of $K_4Fe(CN)_6$ in equal proportions.

Result.—No iron reaction could be demonstrated.

Experiment (8).

After several trials a modified technique was devised, which enabled us to obtain the iron reaction with both hæmozoin and with hæmatin.

Films of hæmozoin and of hæmatin were prepared on glass slides. After drying in the air, a drop of 6 per cent alcoholic solution of HNO_3 was placed on the film, and it was warmed by holding about 9 inches above a non-luminous Bunsen flame. When the first drop of acid-alcohol had nearly evaporated,

another drop was added and the process repeated 3 or 4 times. The films were then treated with a drop of 2 per cent aqueous solution of HCl and a drop of 2 per cent aqueous solution of $K_3Fe(CN)_6$. The preparation was covered immediately and examined under the microscope.

Result.—Numerous blue granules were seen in both the hæmozoin and the hæmatin films. The test was not tried with hæmin.

(iii) Summary.

It has been possible to demonstrate iron in parasitic pigment, when this substance has been acted upon by acid-alcohol (6 per cent HNO_3) to unmask the organic iron of the hæmozoin.

D. AMMONIUM SULPHIDE.

Hueck (1912) used a freshly prepared, pale yellow solution of $(NH_4)_2S$ to unmask iron from hæmozoin. He reports that after such preliminary treatment he obtained a blue reaction by Turnbull's test. Seyfarth (1921, 1926)*, Mayer (1923)* and Kósa (1925) state that they have been able to confirm these results. On the other hand, Lignac (1923) and Glasunow (1925) failed to obtain the reaction by this method.

Seyfarth (1926) concludes, as the result of his work, that hæmozoin contains iron in a state of combination much looser than it should be if identical with hæmatin. It should be noted, however, that no mention is made by this worker of comparative experiments with hæmatin under similar conditions.

Following the technique recommended by Seyfarth (1926), we were unable to obtain a positive result with parasitic pigment. Modifications of this method were, however, successful.

Experiment (9).

Thick smears of malarial pigment were made on glass slides and allowed to dry in the air. A number of these were then immersed in $(NH_4)_2S$ contained in small glass jars. The reagent was freshly prepared and of a pale yellow colour, as recommended by Hueck (1912). The jars were tightly sealed with glass stoppers.

Slides were removed from the jars at regular intervals†, and thoroughly washed with distilled water. They were then treated with a freshly prepared mixture of equal parts of 20 per cent aqueous solution of $K_3Fe(CN)_6$ and 1 per cent aqueous solution of HCl.

Result.—In no instance was a positive reaction obtained with malarial pigment.

* Mayer (1923) states that Seyfarth (1921) worked with the pigment of *P. vivax*, and that the latter author in a personal communication admits that he was less successful in splitting iron from the pigments of *P. malariae* and *P. falciparum*. He apparently must have been more successful in his later efforts, for he states (Seyfarth, 1926) that he obtained a positive reaction with malarial pigment in all the cases investigated by him. Mayer (1923) worked with the pigment of *P. falciparum*.

† The first slide was withdrawn after 2 minutes. During the first half-hour other slides were withdrawn at intervals of 5 minutes, from $\frac{1}{2}$ to 1 hour at intervals of 10 minutes, from 1 to 2 hours at intervals of 15 minutes, from 2 to 4 hours at intervals of 30 minutes, and from 4 to 8 hours at intervals of 60 minutes. A few slides were left as long as 16 hours in direct contact with the reagent.

Experiment (10).

The technique was modified and the smears were not immersed in $(\text{NH}_4)_2\text{S}$, but merely exposed to the vapour of this reagent. It was thought that the vapour would have a slower action on the pigment, and that there would be the additional advantage that the products of the reaction would not be washed away in the fluid.

A layer of $(\text{NH}_4)_2\text{S}$, about 1.5 cms. deep, was placed at the bottom of a glass jar about 12 cms. in height and about 5 cms. in diameter. The smears of malarial pigment were made on microscopical slides, as in previous experiments. These were placed erect in the tightly stoppered jar, so that the films were exposed to the action of the vapour without coming in direct contact with the fluid.

Slides were withdrawn at intervals of 1 hour, washed with distilled water, and then treated with equal proportions of aqueous solutions of $\text{K}_3\text{Fe}(\text{CN})_6$ (20 per cent) and HCl (1 per cent). After acting for 2 minutes the reagents were washed off with distilled water. The slides were then immersed in alcohol for some time, to remove any deposit of sulphur which might be present.

Result.—Smears of hæmozoin exposed to the vapour for 3 hours showed a few greenish-blue pigment granules, *not* haloes. Slides exposed for 5 hours showed many granules with blue reactions, which appeared very similar to those depicted by Mayer (1923) and described by him as 'blue drops'. Some smears were acted on by the vapour for 16 hours before being tested for ferrous iron. In these the whole smear appeared blue to the naked eye, and on microscopical examination it was found that the majority of the granules had completely dissolved. The blue colour appeared to be due, in the last instance, to the diffusion of the iron from the granules into the protoplasmic background of the smear, which was formed by the remains of the parasites.

Some smears of hæmatin were tried by the same method. Considerable difficulty was experienced in preventing these films from being washed off the slides during the different steps of the technique. In the case of malarial pigment no such difficulty was encountered, because the granules adhered on account of their coating of parasitic protoplasm. In the few instances in which we were successful in saving a small amount of the hæmatin films, this exhibited the ferrous-iron reaction when tested with $\text{K}_3\text{Fe}(\text{CN})_6$ and HCl .

The iron reaction was obtained with hæmatin smears after exposure of 4–16 hours to the $(\text{NH}_4)_2\text{S}$ vapour.

Experiment (11).

Hæmatin granules and hæmin crystals were mixed with small amounts of clear, straw-coloured serum separated from the blood of a normal monkey. These mixtures adhered to the slides when made into smears, and this overcame the difficulty mentioned in the previous experiment.

The smears were dried thoroughly in the air and immersed twice in distilled water for about 15–20 minutes on each occasion. This precaution was taken to ensure that any hæmoglobin, which might be present in the serum, was removed. The smears were re-dried and treated with oxalic acid (2 per cent) for 40 hours. They were washed 4 or 5 times in distilled water to remove all trace of acid. They were then exposed to the vapour of $(\text{NH}_4)_2\text{S}$ for 16 hours and tested by Turnbull's reaction.

Result.—In every instance the films of both hæmatin and hæmin developed a blue or greenish-blue coloration. The films of hæmin crystals showed under

the microscope blue crystal-like shapes resembling the original substance. The reaction appeared to occur after about the same period of exposure to $(\text{NH}_4)_2\text{S}$ as with hæmozoin.

Summary.

It has been found possible by a modification of the Hueck-Seyfarth technique to demonstrate the presence of iron, not only in fresh malarial pigment derived from parasites, but also in granules of hæmatin and crystals of hæmin. The reactions were observed not only around the particles but also inside them.

E. SUMMARY AND CONCLUSIONS.

(1) It has been found possible to obtain iron reaction with parasitic pigment, when this has been unmasked by preliminary treatment with such reagents as H_2O_2 , HNO_3 , acid-alcohol and $(\text{NH}_4)_2\text{S}$. Several modifications of the techniques usually advised for this purpose have been devised, with which more constant results were obtained.

(2) These reactions were obtained even after the pigment had been extracted with 2 per cent aqueous solution of oxalic acid for 40 hours. This eliminated any fallacy due to the possibility of an iron reaction caused by the presence of hæmosiderin, or other ionisable form of iron.

(3) Similar results were obtained with hæmatin granules and hæmin crystals.

(4) The negative results obtained with the original acid-alcohol method of Brown (1911) and by the $(\text{NH}_4)_2\text{S}$ method of Seyfarth (1926), suggest that the pigment present in the parasites may not be exactly identical with the tissue pigment used by these workers. This is possibly due to the action of the cells of the reticulo-endothelial system on the latter after phagocytosis, or to the action of fixatives.

(5) It is concluded that malarial pigment derived from the parasites of the circulating blood can be shown to contain iron in a non-ionisable form, and that ionisable iron is probably absent from it.

IV. THE MODE OF ACTION OF UNMASKING AGENTS.

Although many workers have devised methods for the demonstration of organic iron in animal tissues, yet there appears to be some doubt as to the exact means by which the 'unmasking' reagents produce their effects.

Glasunow (1925) has discussed at some length the possible modes of action of acid-alcohol (HCl) and of $(\text{NH}_4)_2\text{S}$, when used for this purpose. He states that these reagents are only solvents, and so any unmasking action would only be possible if they not only dissolve the pigments, but also decompose them so as to set free iron ions. In his experiments he was unable to demonstrate any decomposition of malarial pigment with these reagents. He, therefore, questions the theory underlying the methods advocated by Brown, Seyfarth and Hueck for the demonstration of iron in malarial pigment. He considers that the positive results recorded were due to the undetected presence of hæmosiderin along with the hæmozoin.

The results of our experiments go to show that, after preliminary treatment with acid alcohol (HNO_3) or with $(\text{NH}_4)_2\text{S}$, it is possible to demonstrate iron in hæmozoin, hæmatin and hæmin. These positive results are not due to the

undetected presence of hæmosiderin or other ionisable compounds of iron (*vide* Section II). The mechanism of the process leading to the production of a positive iron reaction, therefore, requires further investigation to reconcile, if possible, these divergent results.

(a) ACTION OF ACID-ALCOHOL.

Glasunow (1925) failed to obtain the Berlin-blue reaction after using acid-alcohol (HCl) for the preliminary treatment of sections of malarial tissue. He was also unsuccessful, when he treated with the same solution pigment extracted from such tissue with an aqueous solution of KOH. We were unable to demonstrate this reaction with hæmatin and hæmin when such acid-alcohol was used [*vide* experiment (7)]. Brown (1911) reports that in his experiments this reaction was helped by the preliminary oxidising effects of H_2O_2 before treatment with acid-alcohol.

Several workers, quoted previously, have found that the action of alcoholic solutions of HCl is not so effective as that of H_2SO_4 or HNO_3 , in unmasking non-ionisable iron in tissues. In our earlier work (Sinton and Ghosh, 1934), it is recorded that the last two mineral acids decompose hæmozoin, but we were unable to obtain any marked decomposition with HCl. This is one probable explanation of the varying results reported with alcoholic solutions of HCl. In the case of tissue pigment, the results may also be influenced by the action of fixatives, or the undetected presence of hæmosiderin.

When HNO_3 , which has a decomposing action on hæmozoin, was used in alcoholic solutions, it was not very difficult to unmask the iron of hæmozoin, or hæmatin. It is recorded in experiment (6) that when hæmozoin is dissolved in acid-alcohol (6 per cent HNO_3), there is (i) a gradual disappearance of the faint absorption bands of acid hæmatin first seen in such solutions, and (ii) that the same solution gives a positive iron reaction with KCNS. These results indicate that alcoholic solutions of HNO_3 , at least, have not only a solvent action but also one of decomposition.

It, therefore, appears almost certain that the unmasking action of acid-alcohol on non-ionisable iron is mainly due to its power of decomposing such compounds.

(b) ACTION OF AMMONIUM SULPHIDE

As pointed out by Glasunow (1925), it is well known that the action of $(NH_4)_2S$ is to reduce hæmatin solutions to hæmochromogen, a compound which does not give the direct iron reaction. There are two possibilities to be considered in investigating the unmasking action of this reagent—(i) that iron can be detached by the action of the dilute acid (HCl) used along with the solution of $K_3Fe(CN)_6$ to produce the Turnbull-blue reaction, or (ii) that $(NH_4)_2S$ *per se* can decompose hæmozoin, hæmatin and hæmin, and that hæmochromogen is only an intermediate stage in the process leading to this decomposition. The latter would indicate that these pigments are first converted into hæmochromogen by $(NH_4)_2S$ and this chromogen is then further decomposed by the same reagent, the iron being removed as ferrous sulphide.

It is recorded (*vide* Plimmer, 1915) that when hæmatin is reduced to hæmochromogen, its iron can be separated from it with comparative ease by the action of acids. This seems to support the first alternative suggestion given above. It appears possible, however, to obtain by experimentation more certain evidence as to which view is more probably correct.

When hæmochromogen is exposed to air, it is quickly oxidised to hæmatin or para-hæmatin, neither of which contains ionisable iron. If, therefore, $(\text{NH}_4)_2\text{S}$ splits off iron from hæmochromogen, then on exposing the pigment so treated to moist air containing a little ammonia vapour, the ferrous iron should be oxidised to the ferric state. Any undecomposed hæmochromogen remaining would be oxidised to hæmatin. If this be so, the pigment should now show ferric iron reactions with KCNS and $\text{K}_4\text{Fe}(\text{CN})_6$ solutions. On the other hand, if $(\text{NH}_4)_2\text{S}$ only reduces hæmatin to hæmochromogen and does not decompose the latter, then on exposure to air the hæmochromogen should be re-oxidised to hæmatin and no iron reaction should occur. As will be seen from the following experiment the former was found to be the case

Experiment (12).

Smears of hæmozoin, hæmatin and hæmin, previously extracted with oxalic acid, were made on glass slides and allowed to dry in the air. These smears were exposed to the vapour of $(\text{NH}_4)_2\text{S}$, as described in experiments (10) and (11). After an exposure of 16–18 hours, the slides were taken out of the vapour, washed twice or thrice with distilled water and partially dried in the air. They were then placed inside small jars containing a few c.c. of dilute ammonia solution (1 part of liq. ammonia to 20 parts of water), and the jars closed. The stoppers were removed for a short time thrice daily to admit fresh oxygen. After 5 days the slides were withdrawn. Each of the films was then treated first with a very small drop of 2 per cent aqueous solution of HCl and afterwards with a similar amount of 2 per cent aqueous solution of $\text{K}_4\text{Fe}(\text{CN})_6$, or with 35 per cent aqueous solution of KCNS only. The smears were immediately covered with a cover-glass.

Result.—The reactions of ferric iron were observed both naked eye and microscopically with hæmozoin, hæmatin and hæmin.

The results of this experiment indicate that $(\text{NH}_4)_2\text{S}$ not only reduces these pigments, but also decomposes them, partially at least, liberating iron as ferrous sulphide.

(c) SUMMARY.

The actions of acid-alcohol and of $(\text{NH}_4)_2\text{S}$ as unmasking agents have been discussed. Experiments have been recorded which appear to indicate that the unmasking action of these substances on non-ionisable iron is due to the decomposition produced.

V. SUMMARY.

(1) The literature relating to chemical tests for iron in malarial pigment has been reviewed in some detail.

(2) Possible fallacies in the recorded results have been discussed, and the measures necessary to avoid such fallacies have been pointed out.

(3) No evidence has been obtained that hæmozoin contains ionisable iron, such as hæmosiderin, when pigment derived from the parasites of the circulating blood is studied.

(4) It has been found possible to demonstrate non-ionisable iron in such parasitic pigment, by using a preliminary unmasking treatment with reagents such as H_2O_2 , HNO_3 acid-alcohol and $(\text{NH}_4)_2\text{S}$. Positive iron reactions were

obtained even after the pigment had been treated with oxalic acid (2 per cent) for 40 hours or more. The latter step was taken to exclude the presence of any ionisable iron compound, such as hæmosiderin.

(5) The possible modes of action of such reagents have been studied, and the results obtained by experimentation discussed.

(6) A close resemblance has been found between the reactions of hæmozoin and hæmatin with the tests employed. These results support the view that these two pigments are very similar and are probably identical.

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ANOPHELINES FOUND NATURALLY INFECTED WITH MALARIA PARASITES IN TRAVANCORE.

BY

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[19th January, 1934.]

DURING routine examination of mosquitoes caught from villages in an intensely malarial tract in the vicinity of Kulasekharam in Travancore, during the period February 1932 to September 1933, four species of *Anopheles* were found infected in nature, namely, *A. jeyporiensis* James, *A. varuna* Iyengar, *A. fluviatilis* James (*A. listoni* Liston) and *A. culicifacies* Giles. The details in regard to these positive findings are furnished in the present article.

Anopheles jeyporiensis.

Three specimens of this species were found infected in nature, all of which were oöcyst infections of the midgut. The first positive specimen was observed in February 1932 (one out of 36 dissected during that month) and showed one large sized oöcyst which was in a nearly mature condition and contained well differentiated sporozoites. The second positive specimen was observed during December 1932 (one out of 68 dissected during the month) and had 17 well developed oöcysts in the gut. The third positive specimen was observed during May 1933 (one out of 15 dissected) and showed a very heavy infection of the gut. There were 80 oöcysts most of which were half-grown; a few oöcysts were in an advanced stage of development. Plate I, figs. 1 to 4 are photomicrographs of oöcysts found in the gut of *A. jeyporiensis*. Dissections carried out during the other months were negative. Taking the total number of mosquitoes of this species dissected during the period of observation, namely, February 1932 to September 1933, three specimens were positive out of 1,988 dissected.

The oöcysts in the positive specimens on examination appeared to be mostly *P. falciparum* judged from the nature of the pigment (Plate I, fig. 4). A few oöcysts were observed which had coarser pigment and these were probably *P. malariae* oöcysts.

It would appear that the relation of *A. jeyporiensis* to malaria transmission had not been definitely known. Covell's summaries (Covell, 1927 and

1931) show that both experimentally and in nature *A. jeyporiensis* was found negative in several large series of observations. Although some workers had suspected on epidemiological grounds that this species might be a transmitter of malaria, definite proof incriminating it was not forthcoming. The positive results obtained in three instances in Travancore are therefore of interest to workers in India. These positive findings in *Anopheles jeyporiensis* James are probably the first records of infection in this species (type form).*

Anopheles varuna.

This species had been previously found infected in nature on only one occasion (Iyengar, 1928) from Bengal where one out of 25 examined was positive for sporozoite infection of the salivary glands. Since then, it had been found to be susceptible to experimental infection with all the three species of malaria parasites (Iyengar, 1933), but no further records were made of natural infections in *A. varuna*.

During the present series of observations on mosquitoes caught from villages near Kulasekharam, one specimen of *A. varuna* was found infected during October 1932 (the only one dissected during the month). Taking the total number of specimens examined during the entire period of observation, natural infection (oöcyst infection of the midgut) was observed in one out of 59 examined. The positive specimen showed 18 well developed oöcysts in the gut (Plate II, fig. 1). The oöcysts observed appeared to be mostly *P. falciparum*; a few oöcysts, resembling those of *P. vivax*, were also seen in the specimen.

Anopheles fluviatilis (A. listoni).

This species had been previously observed infected in nature by several workers. In the present series of observations five specimens were found infected, one of which was a sporozoite infection of the salivary glands while four others were oöcyst infections of the midgut. The positive specimens were observed during different months as follows: three in February 1932 (three out of 52), one during March 1932 (one out of 25) and one during June 1932 (one out of 5). Taking the total number of specimens of this species examined during the period, 5 were positive out of 132 dissected. Of the five positives, one was a heavy sporozoite infection of the salivary glands, and the other four were oöcyst infections with 2, 2, 5 and 55 oöcysts respectively. The infection observed in this species appeared to be chiefly *P. vivax*; a few *P. falciparum* oöcysts, resembling those of *P. falciparum*, were also observed (Plate I, fig. 4).

Anopheles culicifacies.

This species had been previously recorded as infected in nature by many workers. In the present series of observations in Travancore, one specimen

* Recently positive findings in a varietal form of *A. jeyporiensis* have been reported from the Far East. Toumanoff (1931) working in Indo-China observed oöcyst infections of the gut in three specimens out of 50 *A. jeyporiensis* var. *candidiensis* Koidz. (var. *tonkinensis* Tourn.) caught in nature. Feng (1932) reported the finding of oöcyst infection in one specimen of *A. jeyporiensis* (var. *candidiensis* ?) out of 30 specimens examined. Toumanoff (1933) published his further results with the same variety and recorded the finding of natural infections in three more specimens of var. *candidiensis*, in all of which he found sporozoites in the salivary glands.

PLATE I



Fig 1



Fig 2



Fig 3



Fig

EXPLANATION OF PLATE I

- Fig 1 Photomicrograph of midgut of *A. jepponiensis* showing oocysts $\times 263$
 Figs 2 and 3 Photomicrographs of oocysts from midgut of *A. jepponiensis* $\times 580$
 Fig. 4 Photomicrograph of oocyst of *P. falciparum* from midgut of *A. jepponiensis* showing pigment granules in the oocyst $\times 580$

PLATE II.



Fig. 1.

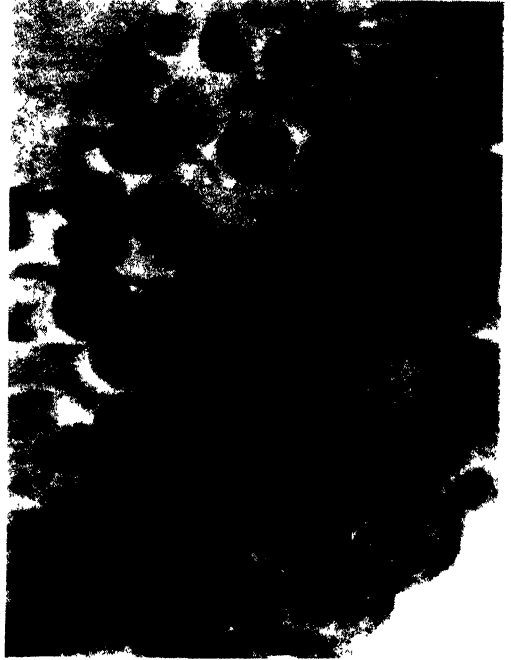


Fig. 3.



Fig. 2.



Fig. 4.

EXPLANATION OF PLATE II.

- Fig. 1. Photomicrograph of midgut of *A. varuna* showing oöcysts $\times 65$.
 „ 2. Photomicrograph of midgut of *A. fluviatilis* showing heavy oöcyst infection $\times 65$.
 „ 3. Photomicrograph of midgut of *A. fluviatilis* showing oöcysts $\times 263$.
 „ 4. Oöcysts in the gut of *A. fluviatilis* showing pigment (one *P. falciparum* oöcyst is situated on the right below and surrounding it are three *P. vivax* oöcysts) $\times 580$.

was found infected during March 1932 (one out of 103 dissected). Taking the entire period together, one showed natural infection out of 984 dissected. The infected specimen showed a sparse sporozoite infection of the salivary glands.

SUMMARY.

During routine examinations of mosquitoes caught from endemic villages near Kulasekharam in Travancore, four species of *Anopheles* were found infected with malaria parasites in nature, namely, *A. jeyporiensis*, *A. varuna*, *A. fluviatilis* and *A. culicifacies*. Sporozoite infections of the salivary glands were observed in *A. fluviatilis* and *A. culicifacies*. In the case of the two other species, heavy infections of the gut with oöcysts were observed and several of the oöcysts were full grown and showed well formed sporozoites.

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RATIO OF SEXES OF SOME INDIAN ANOPHELINES HATCHING UNDER CONTROLLED CONDITIONS IN THE LABORATORY.

BY

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INTRODUCTORY.

IN the course of breeding experiments, one is often struck with the curious phenomenon of the differential emergence of sexes in the adult anophelines. This was first observed by Rees (1901) who found that when mosquitoes are bred in captivity the males, as a rule, hatch out first, and in greater numbers than the females. Nuttall and Shipley (1902) were, however, unable to confirm this finding and asserted that the proportion of males to females had always appeared to them to be fairly equal. In Mauritius, Ross (1911) collected mosquitoes emerging from a swamp under natural conditions, by covering an area with a large net. Of his total of 61 adults taken from the net, 30 were males and 31 females. The figures of Bacot (1915), regarding the emergence of males and females of *Stegomyia* in West Africa, tend to corroborate in the main the observations of Nuttall and Shipley and of Ross. Out of a total of 913 adults of *Stegomyia* bred out by him, there were 427 males and 486 females, the latter evidently being in preponderance.

Stephens and Christophers (1908), however, state that there is a larger proportion of males among the artificially raised mosquitoes. Similar findings are reported by other workers, notable amongst those being Gordon (1922) and Young (1922). Harold (1923) proceeded a step further and asserted that the adults of *Anopheles maculipennis* Meigen emerged in the ratio of three males to one female. This statement was further amplified by Mayne (1925) who remarked that in some of the *Culex* the proportion of males to females has been observed to be six and seven times to one; and the usual maximum anopheline ratio is three to one.

It is hard to reconcile the latter evidence with the observations of workers such as Van Breeman (1920), Lamborn (1922), Russell (1925), Bradley (1926), Buxton and Hopkins (1927) and Boyd (1930), who have all emphasized that

the sexes either emerge in equal proportions, or the females preponderate in nearly all cases.

The accounts referred to above give some idea of the numerical emergences only. There is very little data upon which the sharp decline in sex ratio either way can be explained. It has, however, been suggested by Berkeley (1912) that, if the larvæ be kept supplied with abundant food, the proportion of males is much reduced. Gordon (1922) considered the possibility that the food supply might influence the sexes differently, and investigated the matter. He, therefore, bred out larvæ of *Stegomyia calopus* Meigen on different food supplies, other factors being kept as nearly as possible equal. He observed a mixed batch of ova, hatched and allowed to develop under food conditions which were either adverse or favourable to growth. His findings were that a much greater number of males than females emerged during the first few days and also in the completed experiments.

In view of the contradictory nature of evidence reproduced above, this problem seemed worthy of being reinvestigated particularly with regard to the influence of varying food conditions on the emergence of sexes. Such an investigation was more necessary as no previous experimental evidence was available about the behaviour of Indian anophelines.

Unfortunately Gordon's work (1922) refers only to *Stegomyia* and that too mainly restricts itself to the order of hatching in a mixed batch of ova. It would, therefore, be interesting to know what is the sex ratio in the case of anophelines when adults are reared from the same batch of eggs from the same insect under properly controlled conditions of food supply.

METHOD AND TECHNIQUE.

The initial procedure adopted in my experiments was to hatch out larvæ of the two species of *Anopheles*, viz., *A. subpictus* and *A. annularis*, from a known batch of eggs. These were reared in Petri dishes of standard size (1 inch \times 6½ inches), each containing 200 c.c. of distilled water. For feeding larvæ (i) *Spirogyra*, (ii) pure yeast, and (iii) macerated flies were selected.

(i) *Spirogyra*.

Fresh filaments of the common alga (*Spirogyra*) were taken and dried thoroughly in the sun. These were then pulverized finely and sifted through a closely-meshed piece of muslin. The fine grains thus obtained were then sprinkled on the surface of water. Incidentally this method has proved extremely useful in rearing very young larvæ; the fine particles being readily engulfed and ingested by them*.

(ii) Yeast.

Boyd (1930) recommends yeast as a food for rearing larvæ. Pure yeast† was also sifted through a fine piece of muslin and similarly sprinkled on the water surface. This method was also fairly successful but had the disadvantage that the water had to be frequently changed. This is because the water gets contaminated on account of the yeast cells.

* This method was devised by Lieut.-Col. J. A. Sinton, I.M.S., Director, Malaria Survey of India.

† E. Merck's yeast medicinal dry powder.

(iii) Macerated flies.

In feeding larvæ on macerated flies, specimens of the ordinary house fly *Musca* sp. were caught and crushed so as to reduce the internal viscera to a pulp and cause them to extrude through the body wall. These were given to the larvæ as food. The larvæ feed very readily on this; but here also the water had to be changed very frequently owing to the rapid increase in the ammonia contents of water due to putrefaction of the organic matter.

The details of the experiments actually conducted have been given in the following pages and summarized in the charts reproduced.

DETAILS OF RESULTS OF EXPERIMENTS.

(a) QUANTITY OF FOOD SUPPLY AND RATIO OF SEXES.

It is by no means easy to gauge the actual amount of food consumed by a single larva during various instars. Whatever food matter be substituted, some of its particles must go into solution, with the consequence that only a rough estimate can be made of the amount ingested. But if the larvæ are reared in dishes of standard size with a measured quantity of food matter, we have a method in which it is practicable to control most of the variable factors effectively.

To this end, I selected glass dishes of standard size (1 inch \times 6½ inches) and transferred freshly hatched larvæ from the same batch of ova of *Anopheles subpictus*, each dish containing 200 c.c. of distilled water. Then 0.1 gm. of pulverized *Spirogyra* was sprinkled on each of these dishes. The number of larvæ to be reared was varied, and we placed 30, 100, 200, and over 200 larvæ respectively in each of them. Under these circumstances it was possible to obtain conditions in which some lots of larvæ could secure a maximum food supply, while others out of them received a minimum.

The food was readily consumed in the dishes containing 100, 200, and over 200 larvæ, and fresh additions of pulverized material were made in each case daily so that the larvæ received the same amount of food matter.

The results of these experiments have been summarized in the accompanying charts and also included in Table I. The charts give a clear idea of the order of emergence in both the sexes.

TABLE I.

No. of Exp.	Date eggs laid	Date eggs hatched	No. of larvæ kept	Larval mortality	Pupal mortality	Males emerged	Females emerged	Total emergence
1	7-8-33	8-8-33	30	4	..	13	13	26
2	"	"	100	42	11	21	26	47
3	"	"	200	157	3	17	23	40
4	"	"	over 200	not noted	3	21	23	44

These results show that under the conditions of the experiment the males and females of *Anopheles* emerged in almost equal proportions under varying conditions of food supply*. It was possibly due also to the carnivorous habits

*The heavy larval mortality in experiments 2, 3, 4 was to be expected because of overcrowding and consequent diminished food supply.

of the larvæ, the older ones preying on the comparatively younger. This was a factor which could not be controlled.

The order of hatching in all these experiments has been extremely variable and no evidence is available that males emerge earlier than the females (charts I, II, III, and IV).

(b) RELATIONSHIP OF FOOD-STUFFS TO RATIO OF SEXES.

As the preceding experiment suggested that quantity of food supply does not markedly affect the sex ratio, it was decided to determine whether the nature of food-stuffs has any bearing on the problem. For this purpose a study was made of the relative effect of pulverized *Spirogyra*, yeast and macerated flies on the proportion of males to females in the freshly emerged adults from laboratory-bred larvæ of anophelines.

(i) *Spirogyra*.

It has been found in the previous experiment that sun-dried and pulverized filaments of *Spirogyra* have no marked effect on the inequality of sex ratio. In all the four experiments, the males and females either emerged in almost equal proportions, or the latter more often preponderate numerically. There is no definite order of hatching, either sex might emerge first. The results of these experiments have been graphically illustrated in charts I to IV.

(ii) Yeast.

(A) Freshly emerged larvæ of *Anopheles annularis* were transferred into a glass dish containing distilled water. Pure yeast powder was dusted over it at 8 a.m. every day. The larvæ were allowed to feed on it till 4 p.m., when the water was changed and a fresh supply of distilled water was substituted. No yeast was added to this. Thus the larvæ could feed every day for eight hours. Out of a total of 94 adults which emerged, there were 36 males and 58 females. The number of males which hatched out in the early days of the experiment preponderated, but later the numerical strength of the females was almost double that of the males. The order of hatching is shown in the accompanying chart V*.

(B) In another experiment, natural water from a breeding pool, containing fresh filaments of *Spirogyra*, was substituted for the distilled water used in the previous experiment. The yeast was again used and given for eight hours daily. Fresh *Spirogyra* was also added each time the water was changed. In this case, however, the larvæ had an opportunity of feeding upon other components of the water besides yeast, and they could feed continuously during the whole twenty-four hours. After completion of this experiment an emergence of 188 males against 221 females was recorded—the latter sex evidently being in preponderance (chart VI).

(iii) Macerated flies.

As has been the procedure in previous experiments, newly hatched larvæ of *Anopheles subpictus* were used, but these were reared on a diet of macerated

* The results have been taken from some breeding experiments conducted by Dr. B. M. Roy at the Ross Field Experimental Station for Malaria, Karnal (Punjab). My thanks are due to Dr. Roy for permission to quote these findings.

CHART I

Numbers of male and female Anophelines hatching daily when 30 are kept in a dish of standard size. (*A. subpictus* fed on *Spirogyra*.)

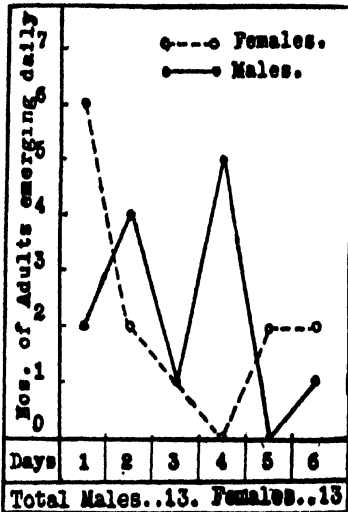


CHART II

Numbers of male and female Anophelines hatching daily when 100 larvæ are kept in a dish of standard size. (*A. subpictus* fed on *Spirogyra*.)

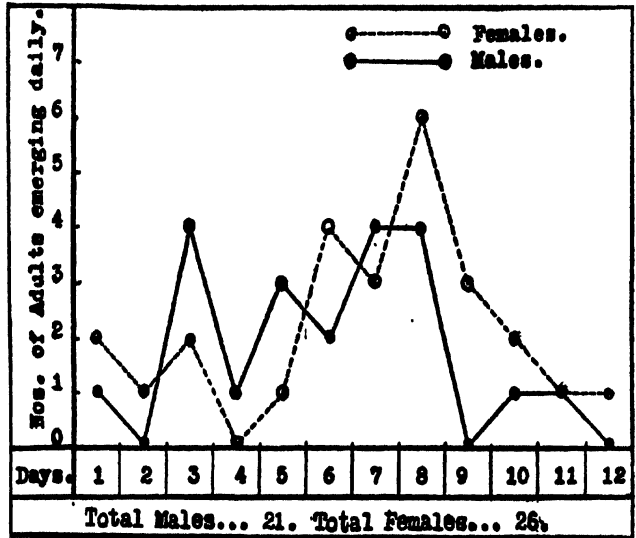


CHART III

Numbers of male and female Anophelines hatching daily when 200 larvæ are kept in a dish of standard size. (*A. subpictus* fed on *Spirogyra*.)

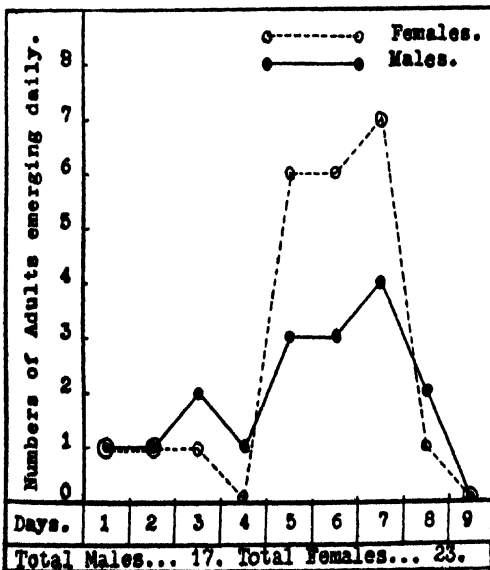
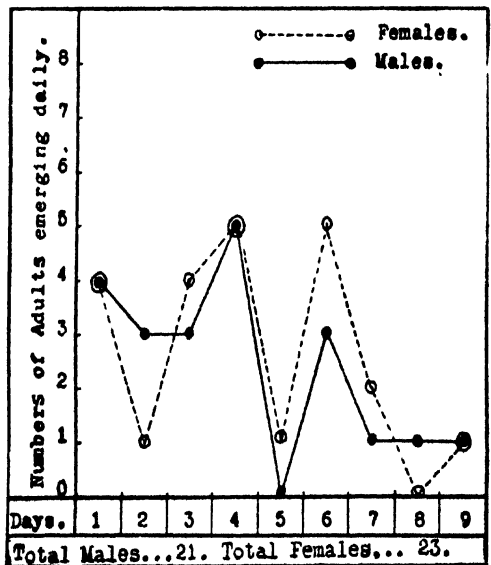


CHART IV

Numbers of male and female Anophelines hatching daily when more than 200 larvæ are kept in a dish of standard size. (*A. subpictus* fed on *Spirogyra*.)



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CHART V

Numbers of male and female *Anophelines* hatching daily from larvæ kept in distilled water and fed on yeast (*A annularis*)

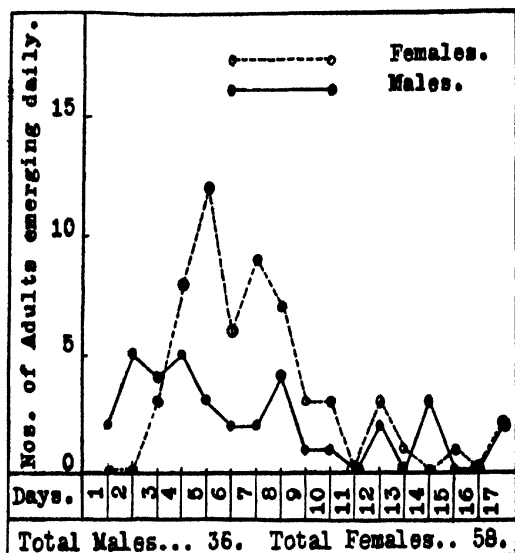


CHART VI

Numbers of male and female *Anophelines* hatching daily from larvæ kept in water from natural breeding place and fed on yeast (*A annularis*)

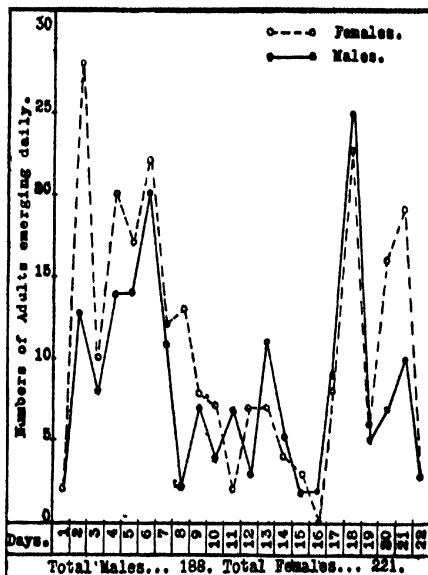


CHART VII

Numbers of male and female *Anophelines* hatching daily from larvæ kept in tap-water and fed on macerated house-flies (*A subpictus*)

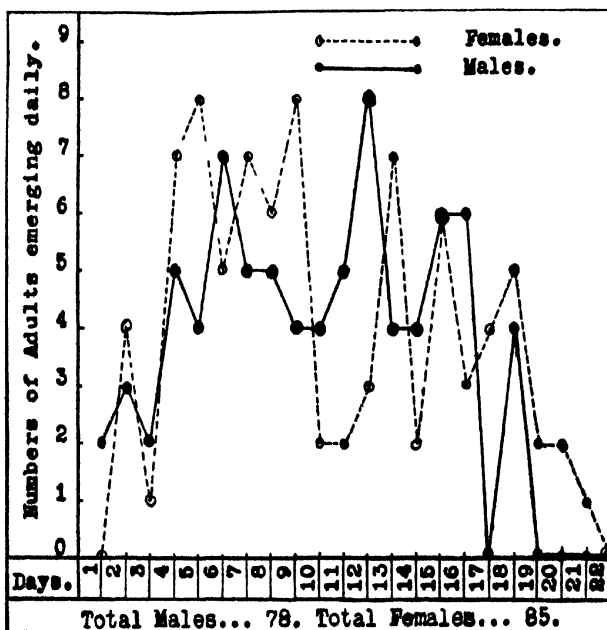
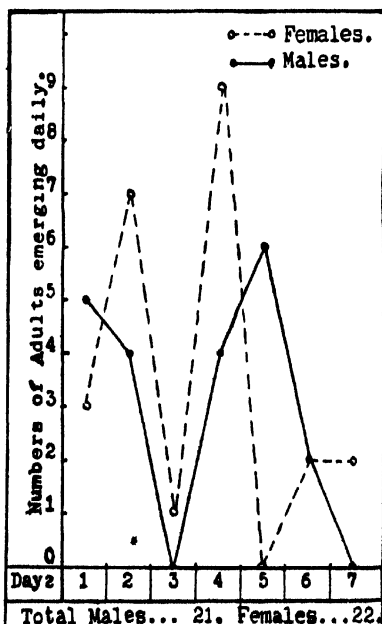


CHART VIII

Numbers of male and female *Anophelines* hatching daily from larvæ fed on macerated house-flies (*A subpictus*)



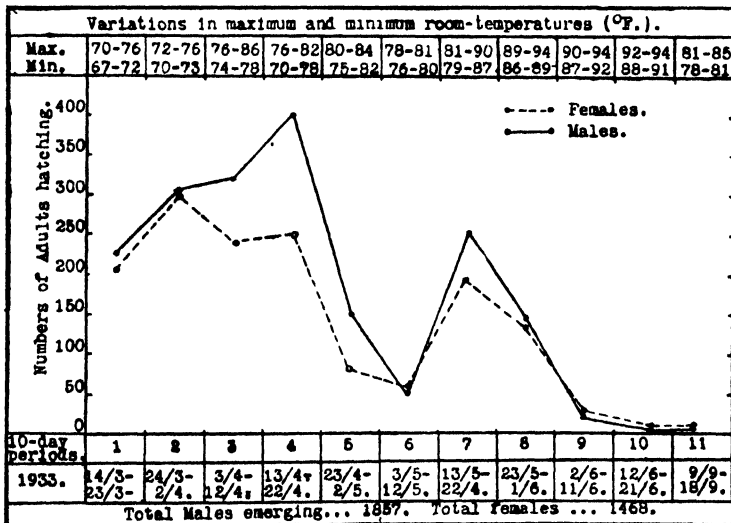
flies, kept in distilled water. The ammonia contents of the water gradually began to rise and consequently it was necessary to change the water daily. This experiment was repeated twice and in each case similar results were obtained. In the first experiment, there was a total emergence of 163 adults, out of which there were 78 males and 85 females. In the second experiment of 43 adults which hatched out, the proportion of sexes turned out to be 21 males to 22 females. The order of hatching is shown graphically in charts VII and VIII. The males and females emerge out not only at the same time but also in almost equal numbers.

(c) RATIO OF SEXES WHEN THE ADULTS HATCH OUT IN NATURE.

It did not appear justifiable to come to any definite conclusions as to the ratio of sexes emerging, merely upon the results of laboratory experiments. Observations were therefore made upon the ratio of sexes emerging in the laboratory from Anopheline larvæ collected from natural breeding places around Karnal. The three common Indian Anophelines, *A. annularis*, *A. culicifacies* and *A. subpictus*, were studied. The seasonal prevalence of these three species varies considerably. For example the first to emerge after overwintering is *Anopheles annularis* and larvæ of this species can be obtained at

CHART IX

Numbers of male and female Anophelines hatching during each 10-day period from larvæ collected in the field (*A. annularis*.)



any time during winter in the plains of the Punjab. This allowed me to commence my observations from the month of March when the maximum temperature in shade does not exceed 85°F., while the minimum ranges about 60°F. After this there is the gradual rise in temperature so that with the advent of summer the maximum temperature shoots up to 100°F. or even more sometimes. During summer, the temperature goes even higher than this and the maximum recorded this year at Karnal was 108°F. During April and May, *Anopheles culicifacies* makes its appearance, and a record was also kept

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of the adult hatchings with respect to sex in this species. The last to appear is *Anopheles subpictus* which was recorded from the last week of May.

CHART X

Numbers of male and female *Anophelines* hatching during each 10-day period from larvæ collected in the field. (*A. culicifacies*.)

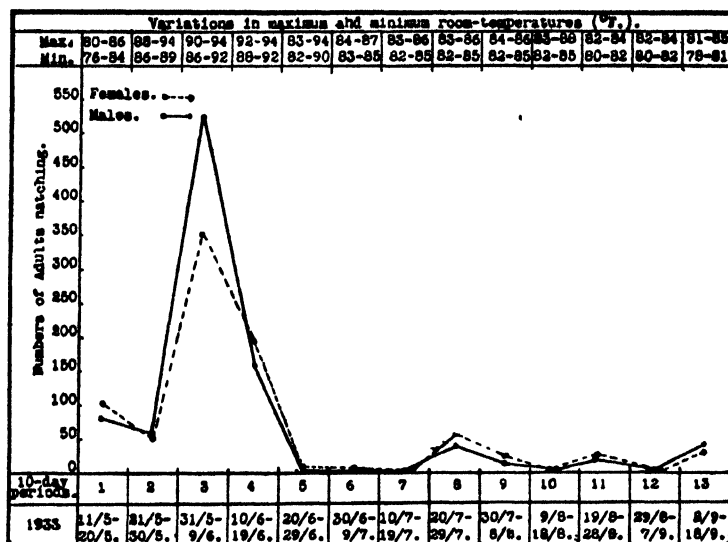
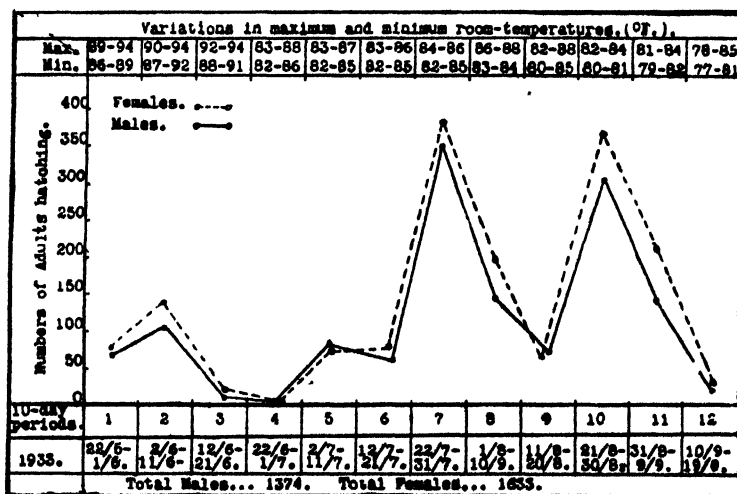


CHART XI

Numbers of male and female *Anophelines* hatching during each 10-day period from larvæ collected in the field. (*A. subpictus*.)



Except in the case of *Anopheles annularis* in which our records cease at the end of June 1933, we have an almost continuous record in the case of the other two species till the middle of September 1933.

The idea of these records was to elucidate, if possible, the preponderance of either sex during any particular season from their hatchings in nature.

In the case of *Anopheles annularis*, there was a total emergence of 3,325 individuals from March to June 1933, out of which there were 1,857 males and 1,468 females. The order of hatching and their numerical strength recorded for every ten days is graphically represented in chart IX. It was noticed that, during March and April, the males emerge in conspicuous majority as compared to the females, but later their numbers are almost equalized.

The preponderance of males over the females is evident also in our records of hatching for *Anopheles culicifacies*. This preponderance was, however, only noted at the time when *A. culicifacies* first hatches out after over-wintering. The differences in sex ratio were almost balanced by an increase in females during later months (chart X). Out of a total emergence of 1,807 adults, we have recorded 947 males against 860 females.

In contrast to the observations cited above, the proportion of males to females was reversed in the case of *Anopheles subpictus*, the species which emerges later in nature, after over-wintering. The total number of adults which hatched out was recorded to be 3,007, out of which there were 1,374 males and 1,633 females. During the earlier emergences the males and females hatched out in almost equal proportions (chart XI) but later the females were in conspicuously large numbers.

DISCUSSION.

There exists a great diversity of opinion regarding the proportions of males to females in mosquitoes which emerge from larvæ bred in the laboratory or in nature. Several experiments were conducted to find out if there is any sharp decline in sex ratio due to the variations in the quantity and nature of food. In most of the experiments I have found that the sexes emerge in equal proportions or the females invariably preponderate. It appears that the quantity of food does not very appreciably affect the sex ratio. To test the effect of different food-stuffs I have reared larvæ on pulverized dry filaments of *Spirogyra*, pure yeast powder, and macerated flies. Under the conditions of these experiments, it was found consistently that the sexes emerge almost equally or the females are slightly more numerous. Thus it appears that food supply used in the experiments has little or no effect in determining an unequal emergence of sexes in the two species of *Anopheles* studied, viz., *A. annularis* and *A. subpictus*. It may be pointed out that females more often emerged in larger numbers than the males—an observation made by several other workers [Van Breeman (1920), Lamborn (1922), Bradley (1926), and others]. This inequality in sex ratio does not appear to depend on the food supply, as is believed by Berkeley (1912).

Gordon (1922) and others considered that usually more males than females hatch out when larvæ are reared in the laboratory. In support of this view Gordon (1922) offered the explanation that when larvæ of *Stegomyia* develop under conditions of maximum food supply, there was much greater number of males than females during the first few days of emergence and also in the completed experiments. He found out that, out of a total of 270 larvæ reared by him, there emerged 142 males and 98 females when maximum amount of food supply was given. Consequently it follows that the ratio of sexes in *Stegomyia calopus* appears to be affected very markedly by food conditions.

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These results under controlled conditions are not confirmed by any of the subsequent workers. This finding is the exact opposite of those recorded by Berkeley (1912) who states that, under similar conditions of food supply, the number of males which hatch out is considerably reduced.

Gordon (1922) has stressed the point that in the order of hatchings the males usually emerge first. In nearly all my experiments under controlled conditions of food supply, no regular order of hatching has been observed, either sex emerging first, or about the same time.

It is very difficult to explain the difference in results obtained by Gordon (1922) and by me. The only plausible view which might explain this difference in results is that Gordon (1922) was using a mixed batch of eggs while I have taken care to experiment on the same batch of ova. It seems probable that the insects are differentiated into sexes as early as the stage of the ovum and therefore the external factors during metamorphosis would hardly account for any inequality of sexes.

CONCLUSIONS AND SUMMARY.

(i) After careful investigation, no evidence has been available to show that quantity of food has an effect on the ratio of sexes in the three species of Indian *Anopheles* studied. The adults emerge in almost equal proportions or in some cases the females preponderate.

(ii) Dry filaments of *Spirogyra*, pure yeast powder, and macerated flies were used to rear the larvæ of *Anopheles subpictus* and *A. annularis*. Again the same conclusion has been arrived at, i.e., the sexes emerge in equal numbers or the females in some cases hatch out in larger numbers than the males.

(iii) The order of hatching is mostly variable—either sex emerging first. At least it does not seem to depend on feeding conditions.

(iv) The seasonal hatchings of the larvæ of *Anopheles annularis*, *A. culicifacies* and *A. subpictus* collected in nature were recorded. This has shown that there is a conspicuous majority of males which emerge during spring after over-wintering. *Anopheles annularis* breeds continuously even in cold weather in the plains of the Punjab. In the case of this species as well similar results have been obtained, the males emerging in larger numbers than the females during March but later, however, the sexes in each species studied, emerged almost in equal proportions.

ACKNOWLEDGMENTS.

This research work was carried out under the supervision of Lieut.-Col. J. A. Sinton, D.Sc., I.M.S., Director of the Malaria Survey of India, to whom my best thanks are due for suggesting this problem and for advice during the course of research, as well as for supplying me with many references incorporated in this paper. I take this opportunity to thank the Indian Research Fund Association for placing at my disposal, through the Malaria Survey of India, laboratory accommodation and material at the Ross Field Experimental Station for Malaria at Karnal.

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ANTI-GAMETOCYTE TREATMENT COMBINED WITH ANTI-LARVAL MALARIA CONTROL.

BY

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AND

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[29th February, 1934.]

1. RESULTS OBTAINED BY ANTI-LARVAL MEASURES ONLY.

THE Railway Settlement of Dangoaposi, the headquarters station of the Amda-Jamda Branch Line of the B.-N. Railway in the Singhbhum District of the Chota Nagpur Division of Bihar and Orissa, has been under malaria control ever since the autumnal epidemic which signalized the first opening of the Branch in 1925. This epidemic, and the initiation of control measures, have already been described by the first author (Senior White, 1928, 1928a). Control has been in force ever since, though it broke down badly in 1927, in which year the first author was working in the Malaria Survey of India, and the second author was wholly employed on the Raipur-Vizianagram Railway Construction. This indicates that the epidemic of 1925 was not an isolated phenomenon, and that, without malaria control, Dangoaposi fully exhibits the hyper-endemicity of the Singhbhum Hills, as described by Christophers (1925), Wats (1924), and Strickland and Chowdhury (1930). The anti-larval measures in force at Dangoaposi have been described and commented on by Knowles (1930) and the Malaria Commission of the League of Nations (1930).

The first author's previous account of the results of anti-malaria work at Dangoaposi covers only two years. It is therefore necessary to show what has been achieved by anti-larval measures alone, prior to the initiation of the experiment which forms the subject of the present paper. The results are given in Tables I and II.

From Table I it will be seen that from the administrative view-point the results of malaria control have been eminently satisfactory. Over the eight years 1925-1932 there has been a decrease in the percentage of the

TABLE I.
Malaria incidence.
Dangoaposi.

Year.	Stationary population.	Cases, stationary population.	Percentage, stationary population, attacked per month.	Days work lost by stationary staff.	Days lost per man, stationary staff, per annum.	Running staff.	Cases, running staff.	Percentage, running staff, attacked per month.	Days lost by running staff.	Days lost per man, running staff, per annum.	REMARKS.
1925	193	579	29.2	650	8.92	14	194	154.0	1,441	111.51	9 months' figures only.
1926	193	158	6.0	178	2.49	14	86	51.2	421	30.05	
1927	193	200	8.6	1,229	16.96	14	118	70.2	951	67.87	Old census assumed.
1928	332	212	5.3	1,099	8.03	73	143	16.3	965	12.95	
1929	373	70	1.6	407	3.30	88	27	2.6	186	2.23	
1930	443	115	2.2	664	3.63	46	41	7.4	302	6.56	
1931	556	46	0.7	263	1.13	45	26	4.8	184	4.08	
1932	663	44	0.5	237	1.16	49	23	3.9	186	3.78	
1933	608	39	0.5	284	1.36	47	20	3.5	186	3.95	Year of present experiment.

stationary population attacked from 29.2 per cent to 0.5 per cent, and of days lost per 'stationary'* employé from 8.92 to 1.16. In connection with the latter figure, however, it has to be borne in mind that the number of days off duty for any disease is to some extent influenced by the number of full-pay days leave an individual has to his credit, as medical leave, in excess of full-pay leave at credit, is on half pay. The figures for the running staff, also tabulated, represent the interaction of several factors. Prior to 1929 such staff worked wholly into uncontrolled areas, where they undoubtedly contracted many infections whilst shunting at the various mine sidings. In 1929 three, and in 1931 a fourth out of six such points came under malaria control, correspondingly decreasing the opportunities of acquiring infection out on the line. The figures show however that with protection of their place of rest even staff so exposed vastly improve in health, though the incidence of malaria amongst them is still 3 to 4 times as high as amongst those who never leave the controlled area, and this is a measure of how much additional infection is picked up on duty outside control limits.

* 'Stationary staff' are those whose duties do not take them outside the controlled area. Dependents are similarly situated, whether their male relatives work wholly within, or work trains outside, the controlled area.

An interesting point emerges from Table I. After the epidemic of 1925, larval control measures were supplemented by quinine all through 1926, 642 oz. of quinine and cinchona febrifuge being supplied to the dispensary that year. As no employé got his 'jungle allowance' for the month unless the Sub-Assistant Surgeon certified that he had carried out any orders given him by the Medical Department (Senior White 1928), it may be taken that the 'chronic relapsers' remaining over from the epidemic were adequately medicated. But after the 1927 epidemic, due to failure of control through lack of supervision, no such additional quinine was supplied, only 76 oz. being supplied in 1928. If now the number of 'lost days' by stationary staff be compared, it is seen that whereas there is a 73 per cent reduction in 1926 over 1925, there is only an 11 per cent reduction in 1928 over 1927. Anti-larval measures in 1926 and 1928 being of about the same standard of efficiency, the differences in percentage reduction after each epidemic can only be attributed to heavy quinization after the first.

TABLE II.
Child spleen rate.
Dangoaposi.

Date.	Number examined.	Nil.	1 finger*.	2 fingers.	3 fingers.	4 to 5 fingers.	Umbilicus.	Beyond umbilicus.	Percentage enlarged.	Average spleen.	Percentage new arrivals since last census.
1-11-25	54	11	11	12	6	4	9	1	79.6	2.22	..
31-10-26	34	23	5	2	4	32.3	0.62	?
20-4-28	32	23	2	4	3	28.1	0.59	?
18-11-28	46	33	5	3	4	1	28.3	0.61	?
24-6-29	44	19	5	6	3	8	3	..	56.8	1.66	?
4-11-29	50	25	4	6	4	4	7	..	50.0	1.58	?
18-6-30	83	65	4	7	3	2	2	..	21.7	0.54	?
11-11-30	70	39	6	8	9	3	4	1	44.3	1.24	?
1-6-31	106	61	7	11	16	5	6	..	42.4	1.20	64
7-12-31	79	46	5	7	4	10	7	..	41.8	1.34	53
2-6-32	99	40	23	24	6	3	3	..	59.5	1.18	37
30-11-32	79	36	8	5	6	11	12	1	54.4	1.87	50
17-5-33	112	65	12	11	8	10	5	1	41.9	1.15	38
6-12-33	120	59	21	18	10	7	4	1	50.8	1.17	23

finger-breadths below the costal margin.

Table II indicates the complete unreliableness of spleen rates in railway colonies, as pointed out by Knowles (1930). Such a spleen rate is markedly affected by transfers, and the bringing in from or sending back to their homes of children. During the course of the present experiment 35 children have arrived in Dangoaposi. Two families of four children each from Raipur (Central Provinces) and Khargpur (Bengal), both non-malarious localities, arrived, and were negative when the rate was taken at the close of the experiment, whereas in a family of five from Santragachi (Bengal) every child had splenic enlargement varying from 1 to 3 f.b.* Of a total of 23 children, who arrived subsequent to treatment, 10, or 43.5 per cent, had varying degrees of splenic enlargement. Children who left, or arrived in, the station, during the course of this experiment, have been omitted from the tabulations. The spleen rate of the 77 children who remained in the station throughout the course of the experiment will be discussed later, in its proper place. It is only this rate which is valid.

In spite, however, of the effect of transfers, it is obvious that for a place which has been under malaria control for eight years, the spleen rate is too high. In fact a comparison of Table II with Table IV suggests that the crude spleen rate of Dangoaposi is being artificially reduced by a constant small influx of children whose average rate is lower than that of the child population of longer residence. In other words, that in spite of the improvement in health revealed by Table I, a considerable amount of infection is still occurring at Dangoaposi. Now in the class of employés (all Indians), found in a minor headquarters station of this type, 'fever' in childhood is taken as such a matter of course that it is but seldom that recourse is had to the dispensary on account of it. Employés such as the Station Master, Loco. Foreman and Permanent Way Inspector do call in the doctor when their children have fever, but the vast bulk of the staff do not, and, as the children are often found playing happily although parasites are present in their peripheral blood, the apparent callousness of the parents is readily understandable. Dispensary attendances for 'fever' are therefore mainly confined to adults. From the point of view of the Railway Administration child sickness appears of little moment, as it does not affect the 'days-lost' figure. Actually, it is really very much otherwise, owing to the general fact that children, in hyper-endemic areas, are the principal gametocyte carriers (Boyd, 1930), which fact has been shown to apply to the Singhbhum Hills by Christophers (1924b). As Senior White and Newman (1932) state 'the staff must be protected against the danger represented by their own children'.

Anti-larval measures at Dangoaposi have been improved in efficiency every year since 1928. The paddy fields are no longer largely left untreated, as described by Knowles (1930), as an investigation of the paddy fields at all stages of plant growth showed:—

- (i) that early in the rains puddles on an unploughed grassy terrace produce *A. culicifacies*;
- (ii) that, when there are high bunds with seepage from the terrace above, *A. culicifacies* is found in considerable numbers in the earlier stages of growth. [Wats (1924) had previously drawn attention to this phenomenon in this district];

* f.b. = finger-breadths below the costal margin.

- (iii) that *culicifacies*, with scattered *pallidus* and *philippinensis*, are generally replaced by *maculatus*, *jeyporiensis*, etc., when the plant is full grown and about to flower, the water beneath now being clear.

On these findings the paddy was placed generally, and not special seepage areas only, under paris green from 1930. The area was increased from 1931, with immediate marked effect on the case incidence, as Table I shows. Further, with growth of population and the building of additional staff quarters more remote from the control centre, the area under control has been extended in several directions from time to time.

To check the adult anopheline incidence in the quarters of the staff, weekly check catches for 20 minutes at each place were instituted during 1933 at four spots distributed over the settlement. The results are given in Table III.

These results indicate how very few anophelines escape the control measures in force, and to our mind are a convincing example of Russell's (1933) dictum, 'there is no example of effective control in the tropics by measures which did not include an attack on anopheline mosquitoes', whilst the results up to 1932 accord with the dictum of the League of Nations (as quoted by Russell) that in experimental investigations only one or another particular method should be employed in a given place, for there has been, since 1926, no more quinine issued to Dangoaposi than suffices to treat those who do get infected, and apply for medicine.

2. ANTI-GAMETOCYTE MEASURES.

The object of the experiment recorded in this paper was to see if even more satisfactory results could be obtained by affording the existing anti-larval method of control the adjuvant of an attack on the gametocyte carriers, since under hyper-endemic conditions every anopheline that successfully evades a control runs a very high chance of carrying infection. We, therefore, decided to see if a single 'blanket' treatment on the children, just prior to the commencement of the transmission season, would effect our object. It was certain that if we attacked gametocytes alone, by plasmochin, in an area where *P. falciparum* is the predominating parasite, there would inevitably be a mass of carriers of the schizogony cycle of this parasite that would remain unaffected. The latter would in all probability go on to gametocyte formation at some period in the season; we therefore considered it necessary to attack the schizogony cycle as well.

We have had quite enough experience of attempting to medicate children with quinine to know that any attempt to subject the undisciplined mob of children that we would be dealing with to even a few days course of quinine would inevitably result in, first, a struggling and yelling resistance by the children themselves, and, in consequence, parental opposition and failure to produce their children. It was imperative that, if success was to be obtained, as far as possible no child should escape treatment. We therefore chose euquinine (ethylhydrocupreine), as our schizonticide, accompanied by plasmochin as our gameticide, the two drugs being administered in a mixture of soft sugar and condensed milk, the latter as advocated by Clemesha (1930).

TABLE
Adults caught in 4 catching stations at

Date.	<i>calicifacies</i> *		<i>fluviatilis</i> *		<i>taruna</i> *		<i>minimus</i> *		<i>aconitus</i> .		<i>jeyporiensis</i> *		<i>subpictus</i> .	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
5-8-33	1	3	0	3
12-8-33	1	3	0	1	0	1	1	2
19-8-33	1	2	0	1	0	3
26-8-33	0	2
2-9-33	1	1	0	1	0	1	0	3
9-9-33	3	0	0	3
16-9-33	2	3
23-9-33	1	3	1	2	0	1
30-9-33	2	2	0	2	1	7
7-10-33	2	2	3	14	0	2	0	1
14-10-33	0	1
21-10-33	0	3	2	7	1	9	0	1
28-10-33	0	2	0	2	0	4
4-11-33
11-11-33	0	1	0	1	0	4	0	1
18-11-33	0	4	0	1
25-11-33	0	7
2-12-33	0	1	0	1
9-12-33	0	1
16-12-33	2	0	0	1	0	3
23-12-33	0	3	0	1
30-12-33	0	1	0	1	0	2
6-1-34
13-1-34	0	1
20-1-34	0	1
27-1-34	0	3
	16	23	3	19	0	7	5	44	0	2	0	27	1	15

III.

Dangoaposi, 20 minutes search each station.

<i>vagus.</i>	<i>maculatus.*</i>	<i>stephensi.*</i>	<i>splendidus.</i>	<i>annularis.</i>	<i>pallidus.*</i>	<i>philippinensis.*</i>	<i>barburostris.</i>	<i>hyrcanus var. nigerrimus.</i>	Number of ♀ carrier s p p. taken. (sp. indicated*).
♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	
4 6	0 1	..	0 1	..	4
1 2	5
..	3
0 1	0 1	2
..	3
..	3
1 2	3
0 1	1 0	6
0 2	11
1 5	18
0 1	0
..	20
0 1	8
..	No catch.
..	1 0	5
..	1 0	5
..	7
..	0 1	2
..	0 1	0
..	0 1	0 1	0 1	0 1	6
..	0 1	0 3	3
..	4
..	..	0 1	..	0 4	0 1	2
..	0 2	0 2	0 1	2
..	0 2	0 3	1
..	0 3	0 4	3
7 21	1 0	0 1	0 11	0 18	1 4	0 1	0 1	1 0	

Close to the railway colony at Dangoaposi, and within, though of course not at the centre of, the controlled area, are two villages, (a) that from which the station takes its name, and (b) a new settlement which has grown up just beyond the railway boundary, composed of low caste people engaged in selling liquor to the railway staff. It was obvious that no results would be achieved if the children of these villages were left untreated, as mosquitoes might be expected to pass with ease between them and the railway colony. Though, therefore, these children, with a few exceptions in the village proper, had nothing to do with the railway, we included them in those treated. These child populations had the advantage of being much more static than that in the railway colony; the results with them are given separately.

Prior to commencing the experiment, the name of every child was entered in a register with particulars of father's name, occupation (in the case of employes), age and sex. It was then examined for its splenic condition, and a thick and thin blood film, on one slide, was taken. Daily treatment columns were then ruled, and filled in as the experiment progressed.

It was of course necessary to have a control to the experiment, without drugs, and this was made in the village of Kolhadi, about a mile from the station. As the nullah that runs through that village is oiled higher up, in the railway control, and the paddy is paris-greened up to less than half a mile from the village, the village is not exposed to the full force of anopheline breeding on all sides. It was however the most suitable village to be taken as a control, and results show that whatever reduction in breeding the railway anti-larval measures may effect, enough remains to bring out the typical happenings in a normal Singhbhum village during a malaria season.

A ten-day course of euquinine was given. As it was uncertain how the children would re-act to plasmochin, and it was imperative that there should be no untoward effects from its exhibition, what should have been a 5-day course given in the middle of the treatment, was generally spaced over 6 days by giving half quantities of the drug on the first two days of the plasmochin period. The doses given were as under :—

Age in years.	AVERAGE DAILY DOSAGE.		TOTAL QUANTITY IN FULL COURSE	
	Euquinine, grains.	Plasmochin, grams.	Euquinine, grains.	Plasmochin, grams.
0- 2 ..	4·25	0·0042	42·75	0·022
3- 6 ..	5·75	0·009	57·5	0·045
7-10 ..	7·4	0·012	74·0	0·06

These doses may be compared with those given by Kligler (1933) in his experiment. His totals, for a 5-day course, were :—

Age in years	Quinine, grains.	Plasmochin, grams.
0- 2 ..	25	0·015
2- 6 ..	37·5	0·05
7-10 ..	50	0·075
11-14 ..	55	0·1

As euquinine is generally considered as having approximately half the therapeutic value of quinine sulphate, our quinine treatment, age for age, was not quite so heavy as Kligler's, but as he drew attention to the decreased efficiency of treatment in children of under 4 years of age, we gave 50 per cent more plasmochin than he did to children in the 'under-two' group.

Distribution was made as follows: overnight the Compounder of the Railway Dispensary weighed out the euquinine into the required number of separate packets containing the various doses. The children were produced in line by their parents, and as they passed along, a Field Assistant placed some sugar on the child's palm. The second author's Laboratory Attendant then emptied a euquinine packet of the required content on to the sugar. The resident Sub-Assistant Surgeon added the plasmochin, which he had previously cut up into quarter tablets of 0.0025 gm. each, a second Field Assistant then added a spoonful of condensed milk, and the child was then told to suck the mixture off its palm, which in every case it greedily did. As the child left the line a caste waterman gave it a drink and poured water on to its palm. The whole procedure was supervised and the doses of each drug called out to those administering it, and recorded, by the second author, seated with the previously prepared record book before him. Such a method of administration, involving the co-operative efforts of six persons, may sound extremely complicated, but it made for absolute certainty that no child escaped getting one or the other drug, and, by giving the Sub-Assistant Surgeon in charge of the plasmochin no other duty to distract him, made it as certain as possible that no excessive dose was given in error.

By this means no less than 94 per cent of 251 children completed the course without fuss or struggle. This number is greater than that shown in Tables IV to VI, which include only children continuously present from beginning to end of the experiment. The average cost of the drugs, sugar and milk per head worked out at Re. 0-9-3.*

As regards untoward pathological effects due to plasmochin, only two cases were observed. One child appeared to be completely intolerant of plasmochin. The other would only tolerate it in small doses, and that after the first doses had caused symptoms.

Case 1.—Female child, Telugu, age 5. Given 0.0025 gm. plasmochin on first day of exhibition of this drug, complained of severe abdominal pain and slight nausea. Accordingly on following day all drugs were withheld. On the third day the same dose of plasmochin was given, followed by the same symptoms. Euquinine alone was given on fourth day without any complaint, but on fifth day, when plasmochin was again added to the dose, the same symptoms supervened. Accordingly this child was made to complete the euquinine course alone, as it was apparent that she could not tolerate plasmochin.

Case 2.—Female child, age 8 (Tagti). This child was given the full age dose of 0.01 gm. plasmochin on the first day of exhibiting this drug. She complained of abdominal pain, but not of nausea. Accordingly, on the following day, plasmochin was withheld but euquinine was given without untoward effects. On third day half the plasmochin dose (0.005 gm.) was given. Abdominal pain was again complained of. On fourth day euquinine alone was therefore given. On fifth day 0.005 gm. of plasmochin was given, and on this occasion no complaint was made, and on the last day of treatment 0.005 gm. was again successfully tolerated, but the course ended with this child having only had half of her total age dosage of plasmochin.

* Plasmochin has since been considerably reduced in cost.

TABLE IV.
Spleen rates.

Place.	PRE-TREATMENT, 17-5-1933.											POST-TREATMENT, 17-1-1934.										
	Number.	Negative.	1 f.b.*	2 f.b.	3 f.b.	4 f.b.	Umb.	Beyond umb.	Percentage.	Average spleen.	Average enlargement spleen.	Number.	Negative.	1 f.b.	2 f.b.	3 f.b.	4 f.b.	Umb.	Beyond umb.	Percentage.	Average spleen.	Average enlargement spleen.
Railway Colony	77	38	9	8	9	8	5	0	50.6	1.4	2.8	77	33	20	10	9	5	0	0	57.1	1.1	2.0
Treated villages	89	31	9	21	13	7	5	3	65.2	1.5	2.8	89	28	24	19	8	6	1	3	68.5	1.5	2.2
Control village	42	8	2	8	7	9	7	1	81.0	2.8	3.4	42	3	4	9	10	10	4	2	92.9	3.0	3.2

* f.b.-finger-breadths below the costal margin.

Both these children were negative for parasites before treatment, but so were many others who took the full course without complaint, so absence of parasites for the drug to act upon is not a causal factor for the symptoms.

Treatment was completed on 14th June. This should have been well in advance of the rains, but in 1933 these started very early, on 12th June, when relative humidity rose to 87 per cent, as against 68 per cent the day previous. This date is taken as the start of transmission for the season, but this only means the first day on which effective sporogony could commence in the mosquito, and there could have been no fresh injections of sporozoites before the close of treatment. Three weeks later, on 7th and 8th July, all the children treated were re-examined for parasites.

Transmission at Dangoaposi probably continued until 1st December, on which date the 8 a.m. dry bulb temperature fell to 60°F. Thereafter the children were left for six weeks for the effects of any relapse to develop, and blood and spleen examinations were again made on 17-1-1934.

TABLE V.
Analysis of seasonal splenic changes.

	PERCENTAGE.		
	Railway Colony.	Treated villages.	Control village.
Enlarged during season ..	19	18	33
Decreased during season ..	31	32	24
Enlargement constant ..	14	26	38
Negative throughout ..	35	24	2

Tables IV to VI show the results of the experiment. From Table I it is apparent that no practical result was achieved. The number of cases occurring in the stationary population, though slightly fewer than in the previous year, is no more than could be expected from the increased efficiency of the anti-larval measures during the season, for as soon as the considerable rise in the adults of the *fluviatilis* group (*vide* Table III) was seen in October, the source of this breeding was traced to an area outside the control limit, which was immediately extended to cover it. Further for a month thereafter, as a special measure, all quarters were sprayed with an insecticide mixture, to deal with the adults that had already emerged.

Table IV shows that the crude spleen rate was not improved, though* the average spleen and the average enlarged spleen show considerable reduction.

Anti-Gametocyte and Anti-Larval Measures.

TABLE VI.
Parasite findings.*

Place.	Date.	Number examined.	Number infected.	Percentage infected.	Number with gametocytes.	Percentage with gametocytes.	P. vivax.		P. malariae.		P. falciparum.		SPECIES DISTRIBUTION PER CENT.		
							All stages.	Gametocytes present.	All stages.	Gametocytes present.	All stages.	Gametocytes present.	P. vivax.	P. malariae.	P. falciparum.
Railway Colony	17-5-33	77	35	45.5	8	10.4	21	7	0	0	14	1	60	0	40
	7-7-33	77	8	10.4	0	0	4	0	0	0	4	0	50	0	50
	17-1-34	77	33	42.9	6	7.8	13	2	1	0	19	4	39	3	57
Treated villages	17-5-33	89	57	64.0	20	22.5	17	4	0	0	39	13	30	0	70
	7-7-33	89	18	20.4	3	3.4	14	2	0	0	4	1	78	0	22
	17-1-34	89	78	87.6	18	20.2	26	2	0	0	51	16	35	0	65
Control village	17-5-33	42	33	78.6	8	26.2	11	1	3	1	19	6	33	9	57
	7-7-33		No examination.												
	17-1-34	42	46	109.5	13	30.9	13	1	3	3	30	9	28	7	65

* A double infection is counted as two infections, hence the apparent impossible percentage in the last line.

Table VI reveals many interesting points, of which the principal are :—

- (i) The immediate effect of treatment was to cause an apparent cure of 53 per cent of the *P. vivax* and 85 per cent of the *P. falciparum* infections, with 82 per cent and 83 per cent of the gametocytes respectively of these species removed. But though the first adult mosquitoes that got through the control must have found very few gametocytes, yet the number of cases in July (8) compares very unfavourably with the corresponding month of 1932 (3) and 1931 (1).
- (ii) By the end of the season the number of infections and of gametocyte carriers in the railway colony is virtually the same as at the start of the experiment. The treated villages, exposed to more adult mosquito infiltration than the colony, show a 23 per cent increase in infection, and a steady gametocyte rate.
- (iii) The percentage of immediate apparent cure is 53 per cent for *P. vivax* and 85 per cent for *P. falciparum* infections. This is contrary to the finding of Kligler (1933) that 'malignant tertian yielded less readily to treatment than any of the other forms', though we cannot find from his Table I figures bearing out this statement. The percentage of apparent cure in his table is, considering the small numbers examined, virtually the same for both species.

It is therefore obvious that, even with a high degree of efficiency in anopheline control, no additional good is to be looked for by a single attack on the gametocyte carriers at the commencement of the season.

3. THE INCIDENCE OF INFECTION IN A HYPER-ENDEMIC AREA, WITH AND WITHOUT ANTI-LARVAL MEASURES.

As it is then apparent that the treatment was entirely without influence on the malaria history of the railway colony during the season of 1933, the data accumulated can be used to study the results of the anti-larval measures in force for the last eight years.

From Table VI we get :—

		Pre-season, percentage infected.	Post-season, percentage infected.
Colony (central)	..	45.5	42.9
Peripheral villages	..	64.0	87.6
Beyond control	78.6	109.5

The percentage of double infections also falls as the centre of the controlled area is approached, as per Table VII below :—

TABLE VII.
Double infections.

	Pre-season.	Percentage of all infections.	Post-season.	Percentage of all infections.	Type.
Colony	1	29	1	33	<i>P. vivax</i> with <i>P. falciparum</i> on each occasion.
Peripheral villages.	2	35	9	11.5	Pre-season both <i>P. vivax</i> with <i>P. falciparum</i> . Post-season 8 <i>ditto</i> , 1 <i>malariae</i> with <i>falciparum</i> .
Control village.	2	66	7	15.2	Pre-season both <i>vivax</i> with <i>falciparum</i> . Post-season 6 <i>ditto</i> , 1 <i>malariae</i> with <i>falciparum</i> .

Tables IV and V indicate the same phenomenon. The full fury of the seasonal attack, as it falls on the typical unprotected Singhbhum village, is very apparent. The outcome is a cent per cent infection rate with a 93 per cent spleen rate. Now Kolhadi village, used as the control experiment, is probably not quite so dangerously situated as regards breeding grounds as the railway colony. In the latter before anti-larval measures were initiated, there were huge engineer-made breeding areas in addition to the natural features of the country, as Plate V of Senior White (1928) clearly shows. It can therefore be taken as certain that without malaria control this station could not be kept open.

The data can also be used in an attempt to apply in a practical manner the theoretical formulæ of Ross (1910). Bentley (1925) gives tables taken from the numerical calculation based on these formulæ by Karl Pearson and Blakeman. The present authors have nowhere seen any attempt to check these results by experimental observations. For this reason perhaps the following exposition, however many fallacies the assumptions, to the making of which they have been forced, may be considered to contain, will not be without some practical value. When it comes to applying these formulæ to field conditions, where what is observed is a mixture of the interplay of infection and relapse over many seasons, difficulties at once arise, and many more observational data than have been accumulated in the course of this experiment are seen to be essential to an adequate treatment of the problem.

We must assume, as stated on page 84 —

- (a) that the state of the railway colony without protection measures would be similar to that of the village of Kolhadi, used as the control experiment in this paper, *i.e.*, that there would be *cent per cent* child infection, if no attempt were made to reduce the anopheline population;
- (b) that using the figures in the last column of Table III, the anophelines are evenly distributed throughout every room in the station. This, as anyone who has done any adult anopheline catching knows, is not a natural occurrence;

- (c) a single room is emptied of anophelines in 20 minutes catching or a factor must be introduced by which to multiply the catches made to get the total population per room. Now the apparatus made by Franz Abel for the Stazione Sperimentale per la Lotta Antimalarica of Italy, which is used in this Department, has, in the hands of an expert, a catching maximum of about 5 mosquitoes a minute, and thus a real expert, such as the first author's Laboratory Assistant, can clear any reasonable infested small room of such a size as 15 feet by 20 feet in 20 minutes. The catcher at Dangoaposi is not of the same proficiency, and we have, by comparison with what the expert Laboratory Assistant in question produces when Dangoaposi is under headquarters inspection, doubled the catches in Table III, last column. Of course any figure used has to be treated as an average for the whole transmission season, whereas the majority of infections most probably occur actually when short-lived peaks in carrier incidence occur, as during October in the table.

Now there are 240 rooms in the railway colony at Dangoaposi. In August to November, both inclusive, the number of carrier-species females taken in 4 rooms per week averaged 6.5 for any week.* The total population of the railway colony living in quarters averages about 600 persons of all ages, therefore in the malaria season of 1933 the average number of carrier *Anopheles* females per head has been :—

$$6.5 \times 2 \times \frac{240}{4} \times \frac{1}{600} = 1.3$$

It is quite impossible to calculate with any accuracy the number of new infections that occurred during the season in the railway colony, Dangoaposi. From Table VI it might be argued that there were none, as the finishing total is less than the starting, or that, on the highly improbable basis that every child not found positive at the second blood examination had been cured, that all 'end-of-season' positives minus those found positive post-treatment are new infections, i.e., 25; or, from Table V, that every child whose spleen either decreased in volume or remained negative during the season, was reinfected or infected. This is 33 per cent of the 77 children examined experimentally. Let us apply this last ratio to the whole 120 children present at a routine spleen examination made immediately at the close of the malaria season, on 6-12-1933. Then 40 children will have been infected. To this must be added 23 adults (non-running staff) who attended the dispensary for malaria† during the season July to November, i.e., 63 infections in all, a rate of 105 per thousand.

Turning now to natural conditions, as revealed by a cent per cent child infection in the control village, Christophers (1924*b*) shows that under such conditions about 50 per cent of the adults are infected. The original spleen rate of Kolhadi resulted in a muster of 81 children. The second author

* This introduces the utterly false assumption that all the carrier species are house haunTERS. We know that *maculatus* and *stephensi* at least rest mainly out of doors.

† Of these 13 (56 per cent) were positive for parasites at first examination. Three more (making 70 per cent in all) were positive when they attended a second time for 'fever'.

considers it reasonable to assume an average of 3 children per pair of adults in the village, which therefore contains $\frac{81 \times 2}{3} = 54$ adults. All the children being infected, and half the adults, this gives an infection rate per thousand of population of 800.

Comparing these figures with the table on page 160 of Bentley (1925), the reduction in the anopheline population in Dangoaposi colony appears to be greater than 75 per cent and less than 90 per cent. A further increase of anti-larval efficiency to the higher figure is capable of reducing the infection rate to zero. Whether such a further reduction is financially possible is quite another matter.

4. ISOLATED OBSERVATIONS MADE DURING THE COURSE OF THE EXPERIMENT.

Before concluding, there are certain isolated observations that can be culled from the accumulated data that are of interest.

1. *Change in parasite species during the season.*—Out of 98 children found positive for parasites both pre- and post-season, in six *P. falciparum* at the start of the season had been replaced by *P. vivax* at its close, whereas in fifteen *P. vivax* had been replaced by *P. falciparum* during the same period. One *P. vivax* was replaced by *P. malariae*, but three *P. malariae* were replaced by *P. falciparum*; as would be expected, *P. falciparum*, in late autumn, was the predominant parasite.

2. *The amount of enlargement an individual spleen can undergo during a single season.*—According to Christophers (1924c) a single untreated infection in a season in a child, producing a normal spleen plus 1 splen, is of the order of 11 cm. A-U measurement. This corresponds to about 1 finger-breadth on the old notation. We have found the following degrees of enlargement at the close of the season in children who commenced the season with the spleen impalpable at the costal margin, and no parasites found in the peripheral blood on original examination in May. We have excluded from the totals no less than 10 children with positive blood but negative spleen, as, though the latter was impalpable at the costal margin, it may actually have already started to enlarge and, in any case, its subsequent enlargement cannot be attributed to a single season's infections.

<i>P. vivax.</i>		<i>P. falciparum.</i>		Mixed infections.
5 enlarged to 1 f.b.*	..	1 enlarged to 1 f b		2 enlarged to 1 f b.
3 " " 2 f.b.		1 " " 2 f.b.
2 " " 3 f.b.		2 " " 3 f.b.
1 " " 4 f b
1 did not enlarge	..	7 did not enlarge.		1 did not enlarge.
Average 1.75 f.b.	Average 0.1 f.b.		Average 1.7 f.b.

* f.b. = finger-breadths below the costal margin.

Now we have shown on page 92 that the *P. falciparum* infections were, in the main, incurred late in the season. By the time our second rate was taken, the full measure of splenic enlargement due to these infections would not have been achieved, amply indicated by the fact that no less than 7 such infections had not apparently affected the splenic volume at all. These figures are therefore not proof that *P. vivax* causes greater degrees of enlargement than the other species. It does however show that, exceptionally, a spleen may enlarge, as the result of a single season's infections, up to 4 f.b. We have examined our total figures to see if there is any differing degree of average enlargement in the sexes in childhood, either due to anatomical differences or to girls possibly being more in the houses than their brothers. On 75 boys and 91 girls we find an average spleen of 1.5 and 1.1 f.b., but the average enlarged spleen is the same, 2.1 and 2.0 f.b. respectively.

In conclusion we have to thank the Superintendent of the Kolhan, the specially administered area in which this station is situated, for so instructing his village headmen that through their co-operation the village children were so easily accessible for examination. We also have to thank Dr. W. D. Speedy, the acting Chief Medical Officer, for sanctioning the application of a small saving on the larvicide budget for Dangoaposi for 1932-33 to the purchase of the drugs for this experiment, and Col. Martin Leake, v.c., F.R.C.S., Chief Medical Officer, for permission to publish this paper.

SUMMARY AND CONCLUSIONS.

1. No permanent increased improvement in either adult or child malaria incidence is achieved by a single blanket treatment attack on gametocytes made just prior to the start of an annual transmission season, in hyper-endemic country.
2. Such a treatment appears to make a lasting impression on the size of the enlarged spleen. The gametocytes are, for a brief period at least, reduced very considerably, both *vivax* and *falciparum* being equally affected, but enough remain to enable transmission to commence as if no reduction had been made, and by the close of the season the number of infections and of gametocyte carriers has risen to the original figure. In the absence of full anopheline control, i.e., towards the periphery of the area treated by larvicides, infections increase during the season, though not to the extent obtaining where no anopheline reduction whatever is in force.
3. In railway settlements the general child spleen rate is so affected by transfers of staff as to be considerably invalidated as representative of malarial conditions in the settlement. The Medical Officer is therefore deprived of a valuable health index in places where, as is common for general sanitary reasons in India, the railway station is placed as far as possible from the town or village it serves.
4. A study is given of a season's malarial history of an uncontrolled village in hyper-endemic country. It results in a cent per cent infection, i.e., every child, and a spleen rate approaching that figure. In the absence of anopheline control the impossibility of maintaining imported labour in such country is demonstrated.

5. A study is made of parasite changes in the peripheral blood during a season, and of the degree of splenic enlargement a single season's exposure to two species of parasite can amount to.

6. An attempt has been made to test observationally Ross's theoretical calculations on the regulation of the amount of malaria by the number of mosquitoes. It is obvious that much more extensive observational data, to replace assumptions, would have to be made before this can be done with any real validity.

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NOTES ON MALARIA IN MYSORE STATE.*

Part V.

THE CONTROL OF ANOPHELINE BREEDING IN BANGALORE CITY AND ITS COST IN MYSORE STATE.

BY

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AND

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[12th February, 1934]

BANGALORE is divided into two separate municipalities, the Civil and Military Station and the City. The city, with which this part of the report is concerned, is the administrative capital of Mysore State and has an area of 11.82 square miles within the municipal limits. According to the 1931 census it had a population of 172,357, with a density of 14,582 persons per square mile. The most densely settled part is the city proper, lying in the vicinity of the Fort. For the purposes of the work here reported the extensions of the city were combined into four areas: The Lal Bagh and Basavangudi areas to the south and south-west of the Fort area; the Chamarajpet area to the west; and the Malleswaram area to the north.

The city averages about 3,000 feet above sea level and has an equable climate, the average maximum dry temperature being 85.2°F. and the minimum 64.6°F., with an average 8 a.m. relative humidity of 78. The highest recorded temperature is 102.4°F. and the lowest, 48.7°F., but the mean daily range is 20.6°F. The year may be divided roughly into four seasons: a cool period (January and February), a hot-weather period (March,

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April, and May), the south-west monsoon (June, July, August, and September), and the north-east monsoon (October, November, and December). The average annual rainfall is 34.06 inches, divided among the four periods, respectively, as follows: 0.47 inches, 6.41 inches, 19.34 inches, and 7.84 inches. Between 1918 and 1932 inclusive the yearly rainfall has been below the average in nine years, above the average in five years, and equal to the average once. For the five years of 1923 to 1927 the rainfall was consistently below the average. The rainfall for 1933 is, by September, considerably above the average.

History of Malaria.

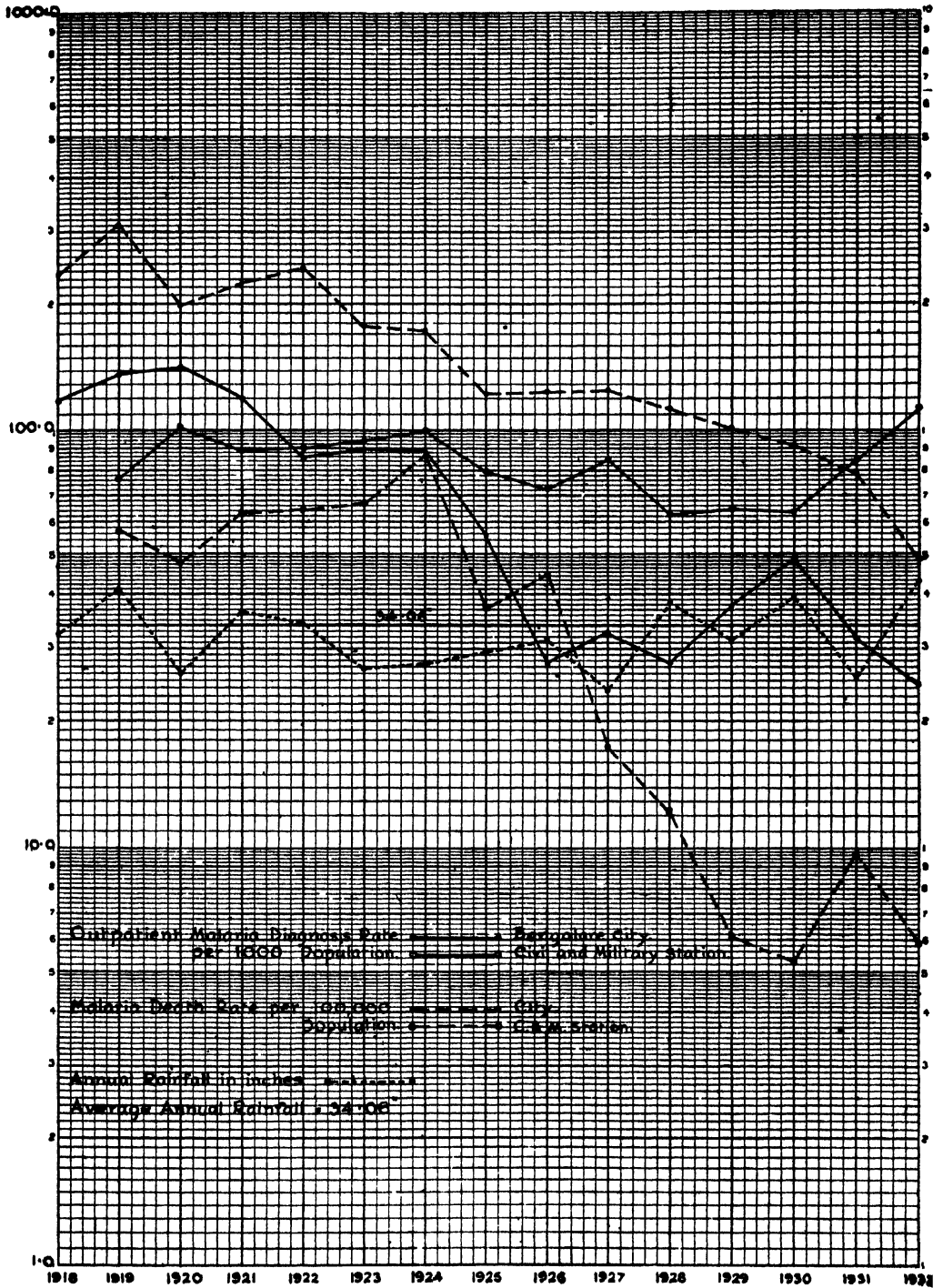
Records can be found of only one previous malaria survey of Bangalore City. These are contained in a report to Government by Fernandes and Subba Rao (1917) and embody the findings of work done between 1st July, 1915, and 30th June, 1916. It is apparent from the wording of this report that it was intended that the work should continue, but no further reports are available. The report covered work carried out in a portion of the present Fort and Lal Bagh areas, during which 3,977 children under 12 years of age were examined and 821 were found to have enlarged spleens, a spleen rate of 20.6 per cent. Blood smears from 13 children were examined but no malaria parasites were found, nor were any infections found in the 236 anophelines that were dissected. These anophelines were of the species *A. subpictus*, *A. culicifacies*, *A. stephensi*, *A. fluviatilis*, and *A. annularis*, but the number of each species dissected is not given. The survey reported 975 wells, in 193 of which (19.8 per cent) *A. stephensi* was found to be breeding. From the spleen rates, it was concluded that the city was malarious at that time, at least in the two areas reported upon, and the writers of the report believed that *A. stephensi*, *A. culicifacies*, and *A. fluviatilis* were the carriers.

Figures are available for the number of diagnoses of malaria in out-patients attending Government hospitals and dispensaries, and for deaths reported as having been due to malaria. Those for 1918 to 1932 inclusive are used in this report. After various methods had been tried, it seemed best to give the figures as rates per 1,000 and per 100,000 of population respectively, using the estimated annual mid-year population to determine the rates. In Graph 1 are given, for both the city and the Civil and Military Station, the out-patient malaria-diagnosis rates per 1,000 population and the malaria death rates per 100,000 population for the years 1918 to 1932; the annual rainfall for each of the 15 years is also included. The city malaria-diagnosis rates may be divided into five groups:—from 1918 to 1920 the rates rose slowly; from 1920 to 1924 they fell to a lower level; in 1925 and 1926 there was a sharp drop; from 1926 to 1930, the rates again showed a tendency to rise, but after 1930 they dropped sharply to the lowest recorded rate in 1932. The death rates from malaria per 100,000 population were about level from 1918 to 1922; between 1922 and 1925 there was a fairly sharp drop to a level which was maintained during 1925, 1926, and 1927; between 1927 and 1931 there was a steady drop in rates culminating in a sharp drop to the lowest rate in 1932.

In the Civil and Military Station the malaria-diagnosis rates remained fairly constant from 1919 to 1924, after which there was a downward tendency until 1930; thereafter, in contrast to the city, the rates rose sharply to the

GRAPH 1.

Out-patient malaria diagnosis rates per 1,000 population, malaria death rates per 100,000 population, and annual rainfall in Bangalore City and in the Civil and Military Station.



highest point for any year, 114.7 per 1,000 population in 1932. From 1919 to 1924 the Civil and Military Station malaria death rates had an upward tendency; from 1924 to 1930 these rates dropped sharply, but since 1930, again in contrast to the city, they have shown an upward tendency.

It should be stated that the diagnosis of malaria, both in out-patients and as a cause of death, is preponderatingly clinical, and that it is probably not possible to compare the absolute levels of these rates from two places. Experience has shown, however, that the rates do bear a relation to the actual malaria present, and that it is probably fair to use their tendencies from year to year as an indication of the trend of the disease itself.

Spleen rates.

A spleen survey of Bangalore City was made in 1927 and has been repeated each year thereafter, the dates of the beginnings of the surveys being, respectively, 22nd June, 6th June, 15th July, 17th July, 17th July, 18th July, and for this year (1933), 19th July. A sample of the children under 12 years of age attending the various schools of the city was examined each year. The spleen rates determined by these surveys for each of the five areas of the city, and for the entire city, are given in Graph 2. For the entire city the 1927 spleen rate was 23.2 ± 1.0 . There was a gradual decline in rates from year to year up to and including 1930, each decrease being significant with reference to its probable error. Between 1930 and 1931 there was a sharp drop in the spleen rates and there has been no significant change since 1931. The rate for 1933, 1.3 ± 0.2 , is significantly lower than any of the rates previous to 1931.

The spleen rates of the five divisions of the city followed much the same course. In the Lal Bagh area there were significant yearly decreases in rates in 1928 and 1929, but the 1930 rate was not different from that of 1929; there was a highly significant drop in rates between 1930 and 1931; since that time there has been no significant change. In the Malleswaram area there were significant declines in rates between 1927 and 1928, 1929 and 1930, and 1930 and 1931, but there has been no significant change since. The spleen rates in the Chamarajpet area show a significant decrease between 1928 and 1929 only, but the 1933 rate was significantly below that for 1929. In the Basavangudi area the significant decreases came between 1927 and 1928, and between 1930 and 1931. In the Fort area there was no significant change until the sharp drop between the 1930 and 1931 rates. There were slight increases (not significant) in the rates of the Lal Bagh, Malleswaram, and Fort areas between 1932 and 1933.

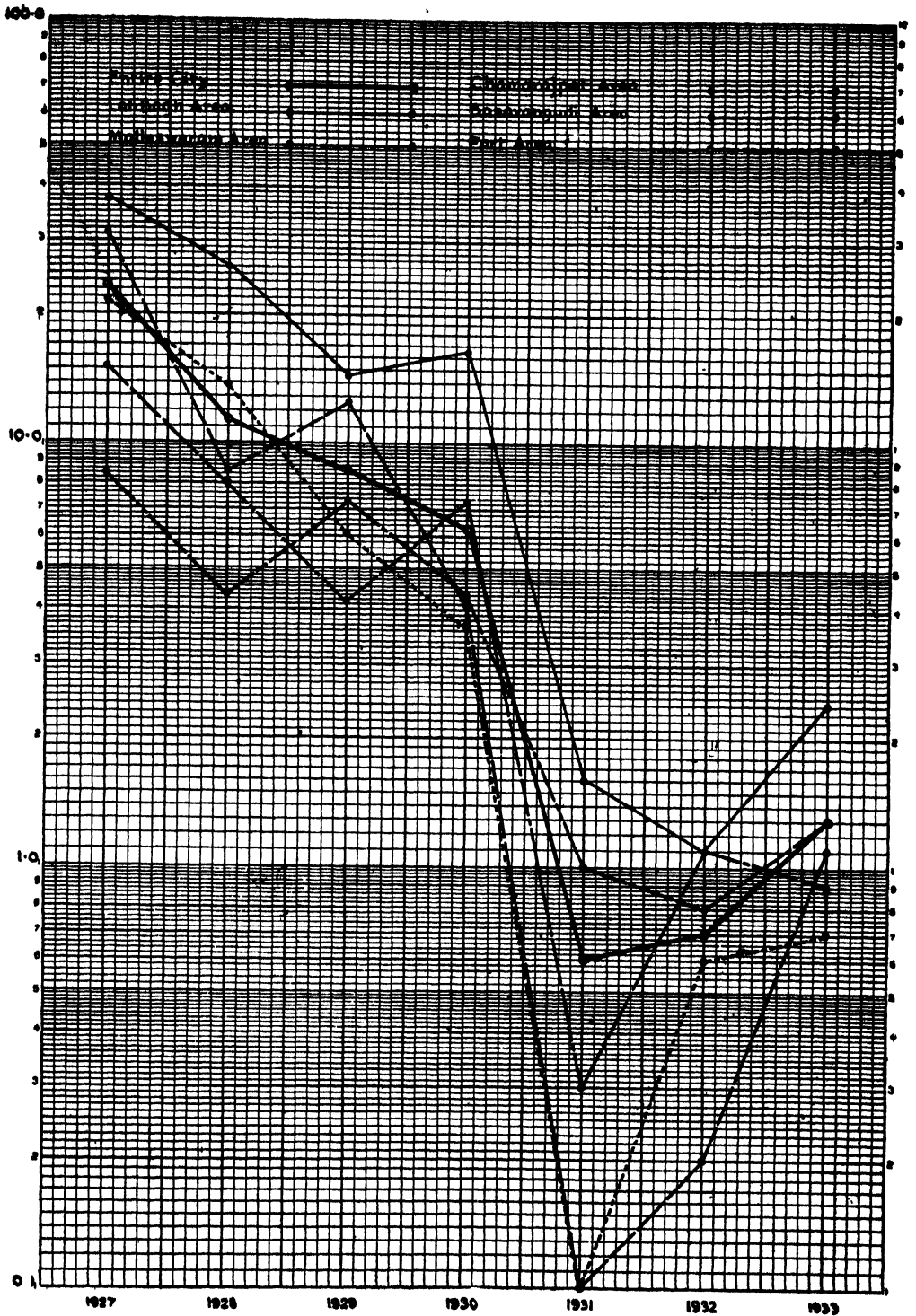
Parasite rates.

Blood smears were taken from about every third child examined in the spleen surveys of 1929 and 1930 and from about every ninth child examined in subsequent years. Since the parasite rates for each separate area of the city were not significant, only the rates for the entire city are given in Graph 3, and the rates for the five areas are shown in two groups of years in Table I.

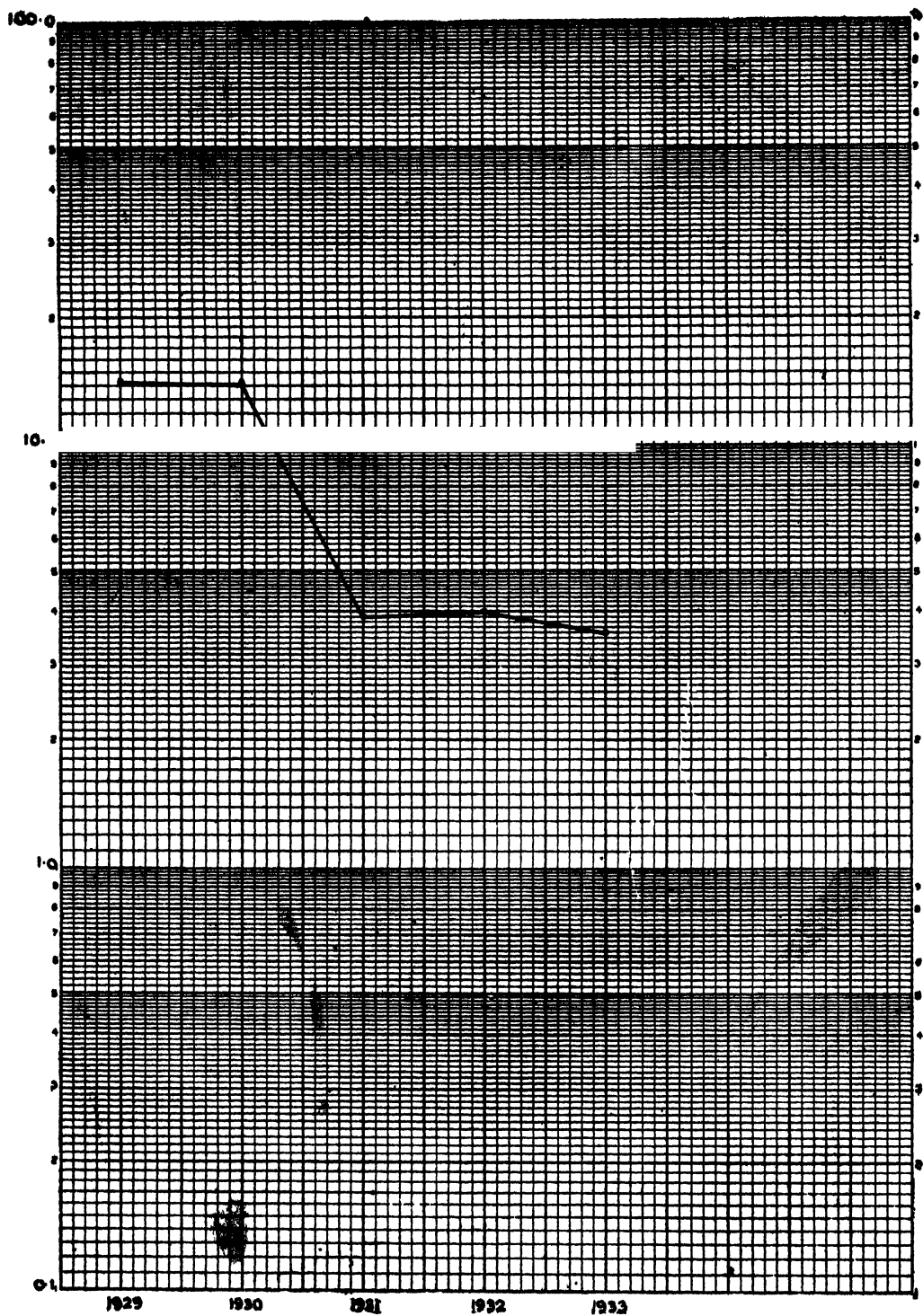
For the entire city, parasite rates for 1929 and 1930 were the same; between 1930 and 1931 there was a sharp drop in these rates to a level which

GRAPH 2.

Spleen rates of five areas of Bangalore City and the entire city, 1927 to 1933.



GRAPH 3.
Malaria parasite rates of Bangalore City, 1929 to 1933



has been maintained in subsequent yearly examinations. With the exception of the Chamarajpet area, the parasite rates for all the examinations of the five areas of the city subsequent to 1930 were significantly lower than the combined rates for 1929 and 1930.

TABLE I.

Malaria parasite rates of five areas of Bangalore City before 1931 and subsequently.

	1929 AND 1930			1931, 1932 AND 1933.		
	Number of children examined.	Number in whom parasites found	Per cent	Number of children examined.	Number in whom parasites found	Per cent
Lal Bagh area	99	21	21.2 ± 2.8	78	6	7.7 ± 2.0
Malleswaram area.	190	36	18.9 ± 1.9	126	5	4.0 ± 1.2
Chamarajpet area.	138	9	6.5 ± 1.4	78	2	2.6 ± 1.2
Basavangudi area.	96	16	16.7 ± 2.6	138	5	3.6 ± 1.1
Fort area	259	29	11.2 ± 1.3	186	5	2.7 ± 0.8
Entire city	782	111	14.2 ± 0.8	606	23	3.8 ± 0.5

Of the 134 blood smears in which malaria parasites were found, 129 were diagnosed as showing benign tertian parasites, three as showing malignant tertian parasites and two as showing quartan parasites. No mixed infections were reported.

Anopheline survey of Bangalore City.

The sources of knowledge of the anophelines of Bangalore City were, in addition to the report of Fernandes and Subba Rao (1917) already mentioned, a report by Iyengar (1926), and records by Covell (1927) of certain specimens, sent to the Malaria Survey of India, Kasauli, at various times. Iyengar examined an extensive seepage under the bund of Sankey's tank in the Malleswaram area, and recorded the following anophelines :

- | | |
|--------------------------------------|--|
| <i>A. annularis</i> van der Wulp. | <i>A. hyrcanus</i> var. <i>nigerrimus</i> Giles. |
| <i>A. barbirostris</i> van der Wulp. | <i>A. jeyporiensis</i> James. |
| <i>A. culicifacies</i> Giles. | <i>A. karwari</i> James. |
| <i>A. fluviatilis</i> James. | <i>A. pallidus</i> Theobald. |
| <i>A. splendidus</i> Koidzumi. | |

In addition, Covell (1927) recorded *A. subpictus* Grassi, *A. tessellatus* Theobald, and *A. stephensi* Liston. In the present survey there were also

found the following anophelines recorded by Covell (1931):—

<i>A. aconitus</i> Donitz.	<i>A. philippinensis</i> Ludlow.
<i>A. jamesi</i> Theobald.	<i>A. turkhudi</i> Liston.
<i>A. minimus</i> Theobald.	<i>A. vagus</i> Donitz.
<i>A. varuna</i> Iyengar.	

It should be mentioned that in this survey neither *A. karwari* nor *A. tessellatus* was recorded as present in the city.

The anopheline breeding places found within the city limits were classified as wells, tanks, and marshes. There are eight tanks in the city, and in four of these *A. culicifacies* was found to be breeding in numbers, while it was rare in the other four. Occasionally larvæ of *A. fluviatilis*, *A. minimus*, *A. aconitus*, and *A. varuna* were found, but never in sufficient numbers to be considered a factor in the malaria situation.

In addition to the seepage in the Malleswaram area, already mentioned, there is an extensive marsh to the west of the Fort area. In both of these places *A. culicifacies* breeding was heavy.

The wells of the city were found to be the breeding places of *A. stephensi*. This species was found to be breeding in other situations once only, when it was discovered in artificial containers near a well. At times *A. culicifacies* was found in garden wells, but practically the only well problem was that of the breeding of *A. stephensi*. The total number of wells in the city was 3,677, of which 3,329 were in connection with houses and 348 were garden wells used for irrigation purposes. Of the 3,329, only about 600 were not in constant use; and of these about 126 were public wells. Farther, of the total number of house wells about 666 were actually inside the houses. *A. stephensi* was found to be breeding in 80 per cent of the house wells, regardless of their situation, use, or disuse. The distance from ground level to water surface in the wells varied from over 25 feet in parts of Basavangudi to about 2 feet in parts of the Fort area.

Control methods.

The control of anopheline breeding presented two problems:—first, that of *A. culicifacies* breeding in tanks and marshes and, second, that of *A. stephensi* breeding in wells. For the control of *A. culicifacies* the Paris green method, described in Part IV of these notes (see footnote, page 95), was used. A 1 per cent mixture of Paris green in road dust and wood ash was found to control the breeding of *A. culicifacies*.

Because of the somewhat precarious condition of the city's water supply, a condition which had existed for some years, and because of certain practices in some communities, there was strong public sentiment against the closing of wells, so that such action has never been found possible. Furthermore, it was not considered that measures for the partial closing and screening of a small opening would have any marked success without a considerable degree of public knowledge and conscience concerning the entire problem, although it is possible that with further public health education such measures might be successful. There seemed to remain only two ways of dealing with the well problem, either to introduce suitable fish or to use Paris green.

TABLE II.

Estimated population, number of out-patients diagnosed as having malaria, number of deaths given as due to malaria, and rates per 1,000 and 100,000 population in Bangalore City and in the Civil and Military Station.

Year	BANGALORE CITY.					CIVIL AND MILITARY STATION.				
	Estimated population.	Number of out-patients diagnosed as malaria.	Rate per 1,000 population.	Number of deaths from malaria.	Rate per 100,000 population.	Estimated population.	Number of out-patients diagnosed as malaria.	Rate per 1,000 population.	Number of deaths from malaria.	Rate per 100,000 population.
1918	103,900	12,400	119.3	246	236.8
1919	109,300	14,977	137.0	339	310.2	115,300	8,786	76.2	66	57.2
1920	114,700	16,447	143.4	220	199.7	117,100	11,800	100.8	56	47.8
1921	120,100	14,412	120.0	271	225.6	118,900	10,535	88.6	75	63.1
1922	125,500	10,747	85.6	307	244.6	120,500	10,760	89.3	78	64.7
1923	130,900	11,690	89.3	235	179.5	122,000	11,429	93.7	82	67.2
1924	136,300	12,029	88.3	236	173.1	123,500	12,233	99.1	108	87.4
1925	141,700	7,737	54.6	176	124.1	125,000	9,967	79.7	47	37.6
1926	147,200	4,046	27.5	184	125.0	126,500	9,213	72.8	56	44.3
1927	152,600	4,980	32.6	192	125.8	128,000	10,808	84.4	22	17.2
1928	158,000	4,514	28.6	181	114.6	129,600	8,047	62.1	16	12.3
1929	163,400	6,174	37.8	164	100.4	131,100	8,497	64.8	8	6.1
1930	168,800	8,151	48.3	155	91.8	132,600	8,471	63.9	7	5.3
1931	174,200	5,542	31.8	137	78.6	134,100	11,261	84.0	13	9.7
1932	179,600	4,347	24.2	87	48.4	135,600	15,558	114.7	8	5.9

TABLE III.

Results of yearly spleen surveys of Bangalore City school children from 1927 to 1933.

Date of beginning survey.	Lal Bagh area.	Malleswaram area.	Chamarajpet area.	Busavangudi area.	Fort area.	Entire city.
22-vi-1927.						
Number examined.	152	221	156	164	166	859
Number with enlarged spleen.	57	69	34	25	14	199
Per cent ..	37.5±2.6	31.2±2.1	21.8±2.2	15.2±1.9	8.4±1.5	23.2±1.0

TABLE III—*concl'd*

Date of beginning survey	Lal Bagh area	Malleswaram area	Chamarajpet area	Basavangudi area	Fort area	Entire city
6-vi-1928						
Number examined	127	188	261	200	161	937
Number with enlarged spleen	33	16	36	16	7	108
Per cent ..	26.0±2.6	8.5±1.4	13.8±1.5	8.0±1.3	4.3±1.1	11.5±0.7
15-vii-1929						
Number examined	145	257	181	213	315	1,111
Number with enlarged spleen	21	33	11	9	23	97
Per cent ..	14.5±2.0	12.8±1.4	6.1±1.2	4.2±0.9	7.3±1.0	8.7±0.6
17-vii-1930						
Number examined	134	295	134	205	327	1,095
Number with enlarged spleen	22	12	5	15	14	68
Per cent ..	16.4±2.2	4.1±0.8	3.7±1.1	7.3±1.2	4.3±0.7	6.2±0.5
17-vii-1931						
Number examined	189	348	147	290	497	1,471
Number with enlarged spleen	3	0	0	1	5	9
Per cent ..	1.6±0.6	0.0	0.0	0.3±0.2	1.0±0.3	0.6±0.1
18-vii-1932						
Number examined	186	415	175	351	515	1,622
Number with enlarged spleen	2	1	1	4	4	12
Per cent ..	1.1±0.5	0.2±0.1	0.6±0.4	1.1±0.4	0.8±0.3	0.7±0.1
19-vii-1933						
Number examined	290	439	274	345	526	1,874
Number with enlarged spleen	7	5	2	3	7	24
Per cent ..	2.4±0.7	1.1±0.3	0.7±0.3	0.9±0.3	1.3±0.4	1.3±0.2

One of the writers (B. A. R.) brought with him from Italy, late in 1928, a small consignment of *Gambusia affinis*. These fish were at first placed in a small fountain and were nearly all lost, but after transfer of the survivors to a pond, and later to certain step wells, they became acclimatized and by the end of 1929 had multiplied enormously.*

It was decided to try the two methods in two parts of the city. *Gambusia* were therefore placed in approximately half the wells and Paris green was used for control in the other half. The fish were also placed in two large tanks late in 1930. They multiplied rapidly and could be found in numbers along the margins of the tanks. Possibly because no clearing of vegetation has been attempted, they have not proved to be a highly satisfactory method of control in this situation and it has been necessary to use Paris green as an additional control.

In addition to routine dipping for larvæ, stations for the catching of adult anophelines were established as a test of the efficiency of the control work. The city was divided into six sections, according to the amount of anopheline control necessary, and in each section 10 catching stations were selected so that there were 60 stations for the city. Of these, 14 were houses only, 24 were combined houses and cattle-sheds, and 22 were cattle-sheds only. These stations were visited once a week for 20 minutes each, and the catch of adult anophelines was subsequently identified and recorded.

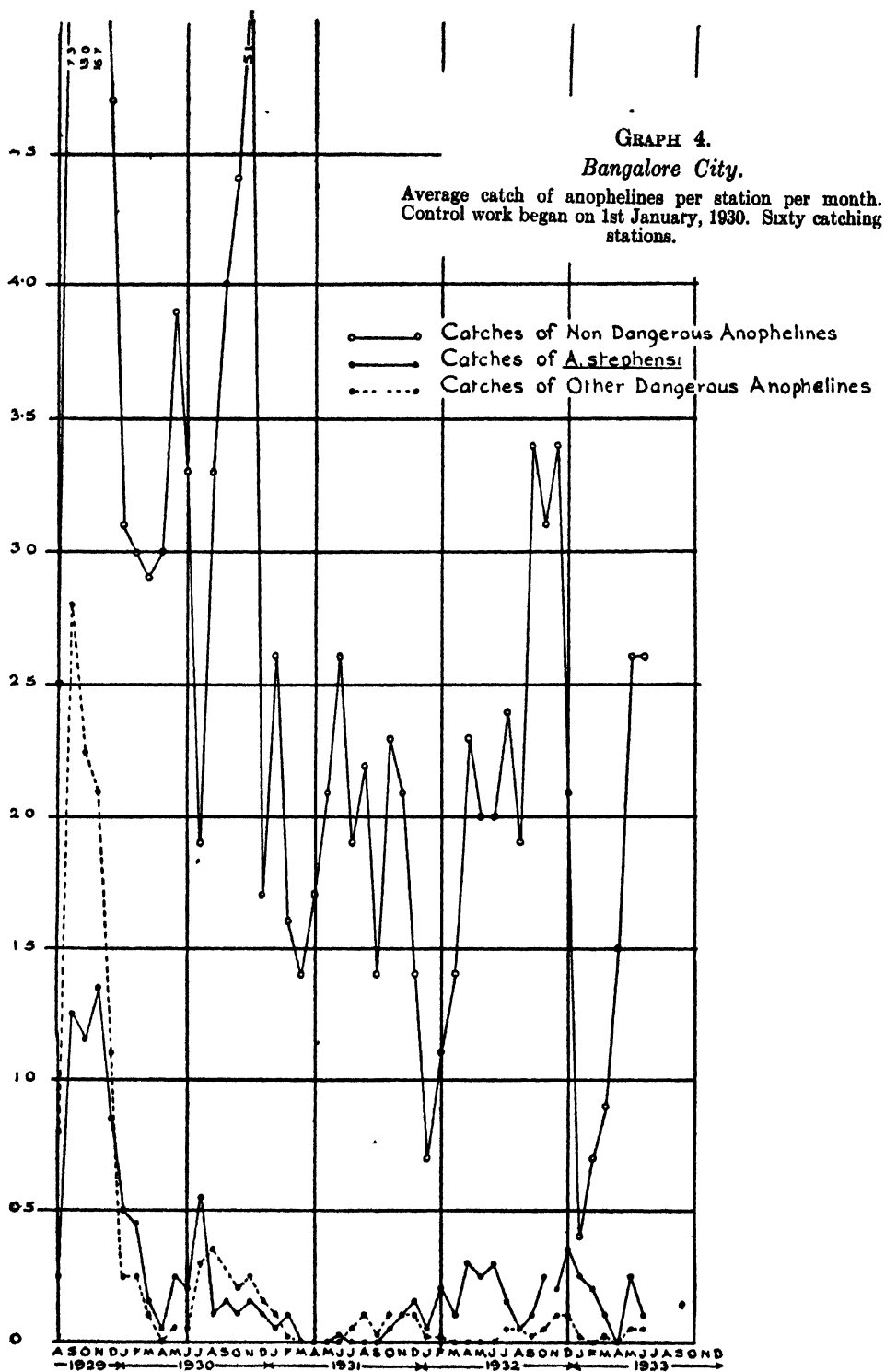
Results of Anopheline control.

After the survey work of the last five months of 1929, control work began in January, 1930, although it was a month or so before the full routine was well established. Graph 4 gives the average catch per station per month of *A. stephensi*, of other dangerous anophelines (mainly *A. culicifacies*), and of all other anophelines, from August 1929 to June 1933. There has been a considerable reduction in the average catches of the non-dangerous anophelines, although no special effort has been made to control their breeding. Such control as was attained was incidental to the control of *A. culicifacies*. In a city like Bangalore the larger part of this catch would consist of *A. subpictus*, which frequently breeds in places in which the only other mosquitoes found are *Culex* spp.

During the last 5 months of 1929, before control measures were instituted, the average catch per station per month of the non-dangerous anopheline species was 8.8; for the same five months of 1930, 1931, and 1932, the average catches were 3.7, 1.9, and 2.8. The increased rainfall of 1932 resulted in some increase in the catches of these species, but at no time has the average catch for the five months been anywhere near half the pre-control catch.

The average catch of *A. stephensi* per station per month during the last five months of 1929 was 0.97. Since then, for corresponding annual periods, it has been 0.12, 0.06, and 0.18. Here again the average for 1932 was higher than that for 1931, a change which will be discussed in a later paragraph.

*It is interesting to note that from an original stock of about 75 fish there are now literally millions available and that successful shipments have been made to various parts of the State of Mysore and to places as far away as Bengal and Burma.



Corresponding figures for the average catches of other dangerous anophelines were 1.81, 0.21, 0.08, and 0.07. There has been no increase in the catches of these species since 1932, and the average monthly catch has not been above 0.12 per station since January 1931. The control of these dangerous species must be regarded as satisfactory.

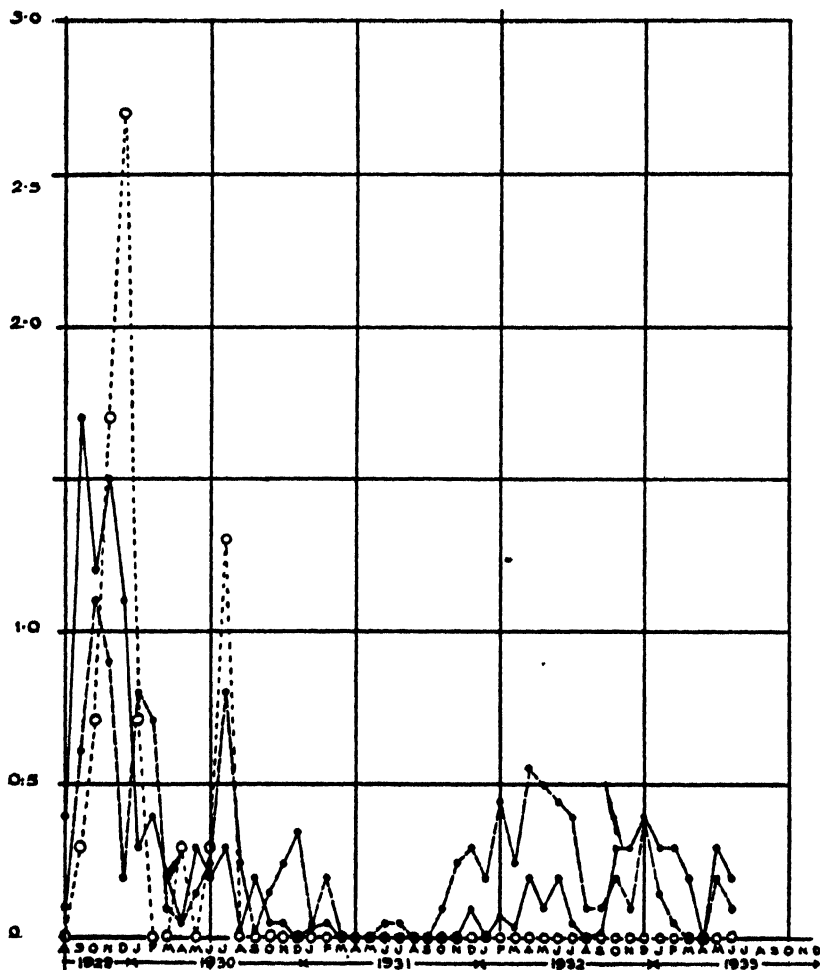
As was mentioned before, at the beginning of the control work some of the wells were stocked with *Gambusia* and others were treated with Paris green. The routine Paris green treatment consisted of 6 puffs with a hand-blower of a 2 per cent mixture of Paris green in road dust, administered once a week. There was naturally some objection to both these procedures at first, but by persistence and tact it was possible to treat all the wells. In fact, of late, there have been complaints when the householder has thought that his well was being neglected. Tests for arsenic in the well water were positive only immediately after the application of the Paris green, and the most delicate tests available failed to show its presence either in deposits from the bottom or in the water of wells which had been treated weekly for considerably over a year.

Graph 5 gives the average catch per station per month of *A. stephensi* in three groups of stations covering areas in which the wells received varying treatments. In an area, covered by 20 stations, the wells were stocked with *Gambusia* from the beginning of the control work. This will be referred to as area A. In area B, consisting of 37 stations, the wells were treated with Paris green from 1st January, 1930, to 31st July, 1931, when they were stocked with *Gambusia*, the change taking some three or four months to complete. In a third area covered by three catching stations, area C, the Paris green treatment continued from 1st January, 1930, to 31st December, 1932, when the change to *Gambusia* control was complete. Since the early part of 1933 all wells in the city have been stocked with *Gambusia*.

During the last five months of 1929 the average catch of *A. stephensi* in area A was 0.58 per station per month, and for the same months of subsequent years it was 0.26, 0.13, 0.18. Corresponding figures for area B were 1.18, 0.64, 0.02, 0.20, and for area C, 1.08, 0.0, 0.0, 0.0. The average catches in area A have been irregular and have seldom come down to zero, except during the period between March and September 1931. In area B the catches came down rapidly after control started and were practically nil from October 1930 to November 1931, when they became irregular, presumably because of the change from Paris green to fish control. In area C, where *Gambusia* were not substituted for Paris green until the beginning of 1933, the catches of *A. stephensi* have been nil since August 1930, although it seems possible that the change to fish control may result in some recurrence of *A. stephensi* catches. The superiority of Paris green to *Gambusia* in the control of *A. stephensi* breeding in wells cannot be questioned. However, since the *Gambusia* method is the cheaper and does result in a very considerable degree of control, it has been substituted for Paris green. The interval between re-stocking of wells with *Gambusia*, determined on the basis of the work of 1930 and 1931, is six months. It seems probable now that to reduce this interval to three or four months would be a wise policy which might result in an increased measure of control. In fairness to the *Gambusia* method it should be mentioned that a considerable number of the wells of area B were

Bangalore City.

C₀..... 3 stations - Paris Green 1-1-30 to 31-12 32 Gambusia since 1-1 33.



dirty and contained a certain amount of debris. This, however, did not apply to area A where the wells were, as a rule, exceptionally clean.

Despite the fact that the rainfalls of 1930, 1932, and the first six months of 1933 were above average, the control of the breeding of *A. culicifacies* and *A. stephensi* is satisfactory, and it seems highly probable that no recurrence of malaria will be possible in Bangalore as long as this control maintains its present efficiency.

The cost of control work.

The cost of control work is an unsatisfactory subject with which to deal, since conditions (salaries, for instance) vary so much, that little application of the information can be made to other areas. However, certain general observations regarding the cost in Mysore State of the control of dangerous anophelines by Paris green may be of interest. The work covered by this discussion has been reported here and in the previous four parts of these notes.

As a rule the Mysore budgets for such control work allow about 72 per cent for salaries of staff, 22 per cent for Paris green and its diluent, and 6 per cent for contingencies. This estimate does not include the initial expenditure for equipment, but does include the cost of replacements. The staff provided for includes a malaria officer, who has had a fair degree of special training in the technical side of the work, one or more technical assistants of sufficient educational qualifications to receive and apply the necessary technical knowledge, and two labourers per division of the area to be controlled, with at least two extra helpers for replacements and extra work.

The work in Mysore has led to the strongly held opinion that successful control work cannot be carried out without an adequate, technically trained staff on full-time duty. In other words, control work under the supervision solely of the general health officer or sanitary inspector will not be a success, but will usually be a waste of any money that may be spent upon it. The entire staff may be under the local health officer, but at least one adequately trained man must be in charge of the antimalarial work on a full-time basis. If the malaria officer has, let us say, two full-time technical assistants, he may possibly spend half his time in other health work, but not otherwise; and under no circumstances can he spend more time on outside work. Anopheline control and tests of its results are of too technical a nature, and require too much expert supervision, to allow of their being placed under the necessarily casual control of officers who have other duties to perform.

From our experience, and under conditions prevailing in Mysore, it seems possible to give the following estimates of the expense of adequate anopheline control with Paris green. In individual villages of from 500 to 2,000 population such control work will cost from 2 to 6 rupees per head of population; from 2,000 to 5,000 population, from 12 annas to 1.5 rupees per head; from 5,000 to 10,000, from 6 annas to 1 rupee per head; in cities above 10,000 population control work would cost from 6 pies to 6 annas per head.* Another possibility in rural areas is presented by a compact area in which there are a number of large villages. In such an area it may be possible to carry out efficient control for from 8 annas to 1.5 rupees per head, depending on the population included, with a staff of one field labourer per village (with extra

* 12 pies = one anna; 16 annas = one rupee.

men for replacement, mixing of Paris green, etc.), one technical inspector for 10 villages, and one malaria officer for a maximum of about 60 villages.

In Bangalore City the budget for control work for the financial year 1932-33 was 5,533 rupees. This amount did not include rent, since the unit was accommodated in municipal buildings used for other purposes, nor did it include the transportation of the diluent used for the Paris green, since this was done by municipal lorries also used for other purposes. As against these items not included, the entire salary of the malaria officer was included, although somewhat over a third of his time was spent in other health work. No charge was made for occasional inspection and supervision of the work by officers of the Mysore State Department of Health whose duty it is to do such work for all parts of the State. This expenditure worked out at just under 6 pies per head of the estimated population and was well under 1 per cent of either the receipts from taxation alone or the total normal receipts from all sources. No expenditures have as yet been made on engineering correction of breeding places, although several plans for such correction have been prepared. Any money spent in this way would reduce the annually recurring expenditure on Paris green and possibly on staff also.

Summary.

Part V of the Notes on Malaria Control in Mysore State describes anopheline control in Bangalore City and discusses the cost of such control in the State by means of Paris green. As judged by the malaria diagnoses in out-patients and the malaria death rates in Bangalore City, there has been a decline in malaria for some years, with a rather sharp drop since 1930 to the lowest points in 1932, while in the adjacent Civil and Military Station there is some evidence of an increase of malaria since 1930. Spleen rates and parasite rates, established by yearly examinations in the City since 1927 and 1929 respectively, both showed a marked drop between 1930 and 1931 to low levels maintained since the latter year. After five months of observations in 1929, the anopheline problem of Bangalore City was found to consist of *A. culicifacies* breeding in tanks and marshes, and *A. stephensi* breeding in wells. Paris green has controlled *A. culicifacies* breeding satisfactorily. The wells were at first treated by stocking half of them with *Gambusia* and treating the other half with Paris green. The latter method is the more efficient in control of *A. stephensi* breeding, but since *Gambusia* control is satisfactory and is also cheaper, this method was adopted for all the wells of the city. A discussion of the cost of anopheline control in Mysore State gives approximate costs per head of population under varying conditions, the cheapest control being in Bangalore City, where the work costs just under 6 pies per head and well under 1 per cent of the normal receipts of the municipality.

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NOTES ON MALARIA IN MYSORE STATE.*

Part VI.

HÆMOGLOBIN AND MALARIA.

BY

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[12th February, 1934]

IN Parts I, III and IV of these notes is discussed the taking of blood films for examination for malaria parasites, and the examining of people for enlarged spleens. As the process was simple and convenient, estimates of hæmoglobin percentages were made by the Tallqvist method whenever blood films were taken. This part of the notes discusses the results of 4,345 hæmoglobin estimates made in the three selected study stations before control work began, also the results of all estimates made in the Nagenhalli area both before and subsequent to the beginning of control work.

Hæmoglobin by age and sex.

Of the total number of hæmoglobin estimates made, 3,460 were of males and 885 of females; 2,150 were of children below ten years of age, 1,491 of persons between ten and twenty, and 704 of those twenty years old and above. Table I gives the average hæmoglobin by age and sex of the 4,345 estimates. For females the average was 70.9 ± 0.2 and for males 72.2 ± 0.1 , a significant difference of 1.3 ± 0.2 in favour of the males. The average for all examinations in these three highly malarious areas was 71.9 ± 0.1 , with extremes of 30 and 95.

There were no significant differences between the averages of males and females by age groups except in the 10-14 and 10-19 year groups, where the

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TABLE I.

*Average haemoglobin by age and sex of all persons examined in three malaria study stations.**

Age groups	MALES.		FEMALES.		BOTH SEXES.	
	Number of persons examined	Average hæmo-globin	Number of persons examined	Average hæmo-globin	Number of persons examined.	Average hæmo-globin
0-4 ..	256	66·8±0·4	192	67·7±0·5	448	67·1±0·3
5-9 ..	1,268	71·6±0·2	434	71·8±0·3	1,702	71·6±0·2
0-9 ..	1,524	70·8±0·2	626	70·6±0·3	2,150	70·7±0·1
10-14 ..	1,096	72·3±0·2	136	74·3±0·4	1,232	72·5±0·2
15-19 ..	241	73·3±0·4	18	72·2±1·5	259	73·2±0·4
10-19 ..	1,337	72·5±0·2	154	74·1±0·4	1,491	72·6±0·2
20 and over ..	599	75·2±0·3	105	68·6±0·8	704	74·2±0·3
All ages ..	3,460	72·2±0·1	885	70·9±0·2	4,345	71·9±0·1

* Estimates made by the Tallqvist scale

female average was the higher, and in the adult age group, where the reverse was true. Considering the males only, there was a steady rise in average hæmoglobin with increasing age up to a high point in the adult group, the average for which was significantly higher than that for any other age group. For the females, the averages rose steadily to a high point in the 10-14 year group and fell off to the average for the adult group. The average for those females 10-14 years old was significantly higher than those for the other ages except the groups 15-19 and 10-19, while the adult average was significantly below that of the 10-19 year group, but not different from that of the 0-9 year group.

It is of interest here to note that, as reported in Part III, a distinct lowering of malaria parasite rates was found to occur with advancing age, and there was no difference between the sexes except in the adult group where the females had the lower rate. Spleen rates of the youngest age group showed a tendency to be lower than those for older age groups, but apart from this there was no marked change in spleen rates with age, and rates for females

were lower than for males in children under ten and in the 'all examinations' group. The average hæmoglobin estimations, as recorded in this part of the report, are what might be expected from some of the spleen and parasite findings, but not from others.

Average hæmoglobin and palpable spleens.

For a consideration of the relation between the average hæmoglobin and palpable spleens it seemed best to limit other variable factors. In Table II, therefore, are given figures for the age group 0-9 of males only. These persons are divided into two groups, (a) those in whose blood films malaria parasites were found at the time of examination, and (b) those in whose blood films no parasites were found. The method of classifying spleen sizes was described in Part III of this report.

TABLE II.

Average haemoglobin, by spleen size and result of blood film examination in 0-9 year old males.

Spleens.	MALARIA PARASITES NOT FOUND IN BLOOD FILM.		MALARIA PARASITES FOUND IN BLOOD FILM.	
	Number examined	Average hæmoglobin	Number examined	Average hæmoglobin.
Not palpable ..	305	72.5±0.3	62	71.3±0.6
Spleen sizes P and 1 ..	363	72.6±0.3	193	70.5±0.4
2 and 3 ..	276	70.5±0.4	248	68.4±0.4
4 and 5 ..	19	66.1±1.7	12	55.0±1.3
All palpable spleens ..	658	71.5±0.2	453	68.9±0.3
All examinations ..	963	71.8±0.2	515	69.2±0.3

There was no significant difference between the average hæmoglobin of those persons whose spleens were not palpable and those with the two smallest of the enlarged spleen sizes, in either the blood negative or blood positive groups, but in both classes the hæmoglobin average was significantly lower for the larger spleen sizes. In the group in whom no parasites were found, there was no significant difference between the average hæmoglobin of those whose spleens were not palpable and of all those with palpable spleens, but this was not true of those in whom parasites were found. The difference, in that case, between 71.3±0.6 and 68.9±0.3 was 2.4±0.7, which is significant.

Average hæmoglobin and malaria parasites.

Further reference to Table II will show that the average hæmoglobin of persons in whose blood malaria parasites were found at the time of examination were significantly lower than those for the other group. This was true for each spleen class with the exception of the 'spleen not palpable' class. For all persons examined, those with parasites had an average hæmoglobin of 69.2 ± 0.3 while those without parasites averaged 71.8 ± 0.2 , a highly significant difference of 2.6 ± 0.4 .

Table III gives the average hæmoglobin of males of the 0-9 age group by species of malaria parasite found and by spleen classes.

TABLE III.

Average haemoglobin of males of 0-9 years in species of malaria parasite found and by spleen classes.

Spleens.	BENIGN TERTIAN.		MALIGNANT TERTIAN.		QUARTAN.		MIXED INFECTIONS.	
	Number examined.	Average hæmo-globin.	Number examined	Average hæmo-globin.	Number examined.	Average hæmo-globin.	Number examined.	Average hæmo-globin.
Not palpable	27	70.9 ± 0.9	5	65.0 ± 1.7	29	72.6 ± 0.8	1	75.0
Spleen sizes P and 1	94	71.3 ± 0.6	28	71.1 ± 1.3	66	69.4 ± 0.7	5	69.0 ± 2.2
2 and 3	142	68.4 ± 0.6	22	68.2 ± 1.7	72	68.3 ± 0.7	12	69.2 ± 1.9
4 and 5	9	58.3 ± 2.5	0	..	3	65.0	0	..
All palpable spleens.	245	69.1 ± 0.5	50	69.8 ± 1.0	141	68.8 ± 0.5	17	69.1 ± 1.5
All examinations.	272	69.3 ± 0.4	55	69.4 ± 1.0	170	69.4 ± 0.4	18	69.4 ± 1.4

No significant variation in their effects on the average hæmoglobin could be found between infections with the three species of parasites and any of the mixed infections, either in those with all sizes of palpable spleens or in all persons examined. Although there were only five persons without palpable spleens found to be infected with malignant tertian, it may be of interest to note that the resulting average hæmoglobin of 65.0 ± 1.7 was significantly lower than that of those infected with either benign tertian or quartan, and also below the average of those in whom no parasites were found.

The lower average hæmoglobin found in those with malaria parasites in their blood were largely due to infections with the benign tertian and quartan species, possibly because of the small numbers of persons with malignant tertian and mixed infections.

Average hæmoglobin as affected by malaria control work.

If there be a relation between hæmoglobin, splenic enlargement, and the presence of malaria parasites in the blood, an increase of average hæmoglobin should be demonstrable subsequent to malaria control work. In Nagenhalli village, spleen, blood film and hæmoglobin estimates have been made in twenty-seven different months between January 1929 and July 1933, the first twelve examinations being made before the beginning of control work in January 1930. The spleen rates, parasite rates and average hæmoglobin of these 27 examinations are given in Graph 1. Both the spleen and parasite rates have dropped very considerably since the control work began, and there would seem to have been some increase of average hæmoglobin during this period.

Up to the date of writing, examinations had been made in Nagenhalli village in February, May and July 1933, and the average hæmoglobin of 128 persons examined in these three months was found to be 77.7 ± 0.4 . The average hæmoglobin of 115 persons examined during the corresponding three months of 1929, before control work began, was 67.7 ± 0.6 . The increase of 10.0 ± 0.7 is highly significant. In three peripheral villages close to Nagenhalli, but only partially protected by the control work (*see* Part IV of these notes), corresponding average hæmoglobins were 74.4 ± 0.6 and 68.5 ± 0.5 , a significant increase of 5.9 ± 0.9 . The difference between these two increases is 4.1 ± 1.1 , which is also significant and in favour of the more thoroughly protected village in which the spleen and parasite rates are also more significantly lowered. In Nagenhalli village there was no significant difference between the average hæmoglobin of all examinations in 1930 and in 1931, but the average of 1932 was significantly higher than that of 1931, and that of 1933 than that of 1932.

In Part I, mention was made of variations of spleen and parasite rates in three groups of months. These groups were (a) February, March, April and May; (b) June, July, August and September; (c) October, November, December and January. The spleen rates for groups (a) and (c) were significantly higher than for group (b), but did not differ from each other; the parasite rate for group (a) was significantly higher than those of groups (b) and (c) which were not different. The average hæmoglobin for these three groups of months were 66.9 ± 0.7 , 69.9 ± 0.7 , and 71.9 ± 0.8 . The average for group (a) was significantly lower than either of the other two averages which did not differ significantly from each other.

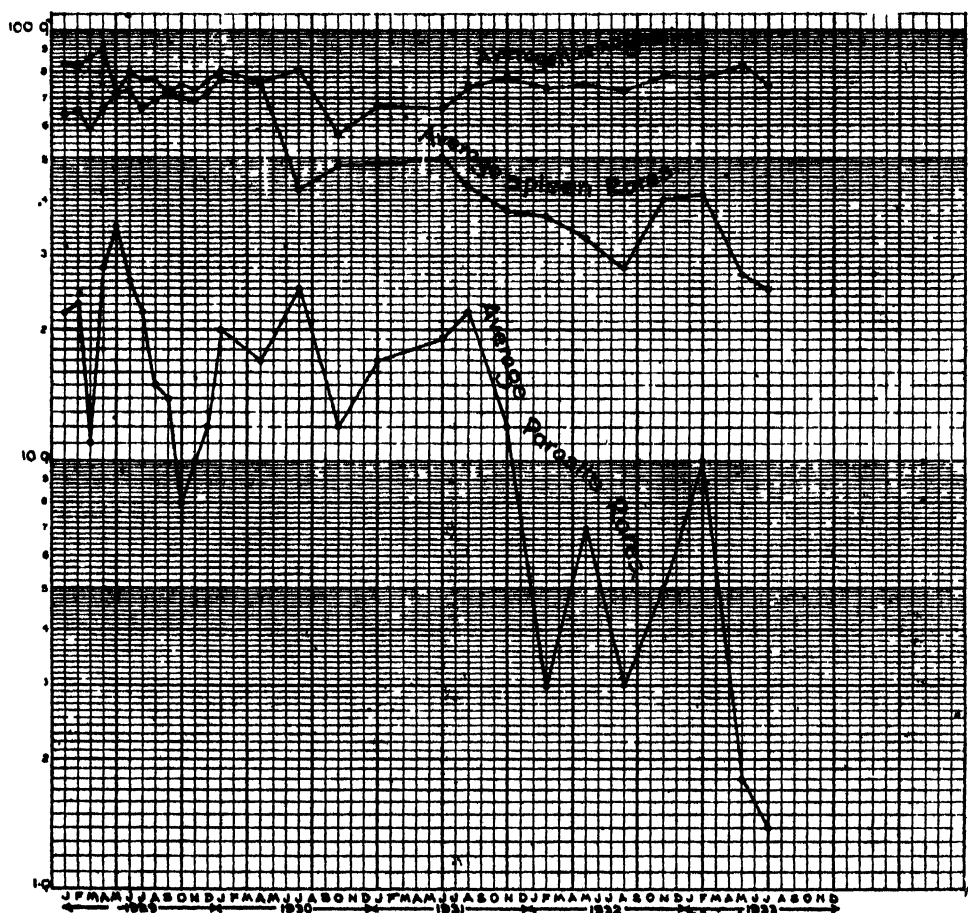
Using the 27 observations of Graph 1 in correlation tables, the following coefficients of correlation were obtained:

For spleen rates and parasite rates,	$r = +0.62 \pm 0.08$.
For spleen rates and average hæmoglobin,	$r = -0.46 \pm 0.11$.
For parasite rates and average hæmoglobin,	$r = -0.21 \pm 0.13$.

The average hæmoglobin was to some extent related to the spleen rates in these observations, the hæmoglobin going up as the spleen rates went down. No significant correlation could be demonstrated between average hæmoglobin and parasite rates. Partial correlations did not improve any of the relationships.

GRAPH 1.

Spleen rates, malaria parasite rates and average hæmoglobin of all persons examined in Nagenhall village. By months from January 1929 to July 1933 inclusive



It must be mentioned that the member of the staff reading the Tallqvist scale for hæmoglobin estimates may have been biased by the result of the previous examination of the spleen, but that he had no means of knowing whether malaria parasites were present or absent. It is thought, however, that this bias could not have been great, since so many of the average hæmoglobins agree with theoretical considerations of which the staff could have had no knowledge.

All of the significant variations of average hæmoglobin mentioned in this report have been considered solely from the statistical viewpoint. There have been no means of judging what the variations meant in the general health of the people concerned. It would seem probable, however, that such an increase in average hæmoglobin as that found in Nagenhalli subsequent to control work, must have meant a general improvement in the health of the population of the village.

Summary.

Part VI of the Notes on Malaria in Mysore State deals with hæmoglobin estimates by the Tallqvist scale, made at the time of the taking of blood films. It was found that (a) males had a slightly higher average hæmoglobin than females, and (b) while in the males the average increased progressively with each rise in age grouping to a maximum in adults of twenty and over, in females the maximum average was in the 10-14 year age group. This finding corresponded more closely to the age relationships of parasite rates, as given in Part III, than to findings for spleen rates.

In males 0-9 years of age in whose blood films no malaria parasites were found, there was no significant difference between the average hæmoglobin of those with and without palpable spleens. There was, however, a significant difference in these averages in the case of those showing parasites in their films. In both the blood positive and blood negative groups the average hæmoglobin went down progressively with increasing spleen size.

The average hæmoglobin in the blood positive group of males of the 0-9 years of age was lower than that of the blood negative group for each class of palpable spleen and for all examined. This was largely due to infections with the benign tertian and quartan parasites, but the average hæmoglobins of those found to have the various species did not differ from each other.

In Nagenhalli village there was, subsequent to control work, an increase of average hæmoglobin which was significantly greater than the increase found in neighbouring partially protected villages. In twenty-seven monthly observations made since January 1929, the average hæmoglobins were significantly correlated, with a negative sign, with the spleen rates but not with the parasite rates.

The increases and decreases in average hæmoglobins were statistically significant, but there was no way of judging what this might mean for the general health of the people concerned.

MALARIA IN SIND.

Part IX.

MALARIA IN SUKKUR DISTRICT.

BY

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INTRODUCTION.

PERIOD AND SCOPE OF SURVEYS.

THE investigations on which the present paper is based were made during six visits to the district, which were made during the period 1927-1930. A severe regional epidemic of malaria occurred throughout Upper Sind in the autumn of 1929. The first three surveys were made before the commencement of the epidemic, the fourth during the early stage of the epidemic, and the remaining two during the first year of the post-epidemic period. The first two surveys, in April 1927 and January 1928, were carried out by Young and Majid (1930), and the remainder by the junior author of the present paper.

The areas visited are situated entirely to the south of the Indus, with the exception of Sukkur town and certain neighbouring villages in Sukkur taluka. Observations carried out in Shikarpur and the neighbouring villages have been dealt with in a separate paper (Covell and Baily, 1932).

The names of the talukas visited, and the periods at which the surveys were made are given below :—

Inter-epidemic period.

1st Survey—Sukkur, Rohri. April 1927.

*2nd Survey—Ghotki, Pano Akil. January 1928.

3rd Survey—Pano Akil. 27th to 30th October, 1928.

Early epidemic period.

4th Survey—Ghotki, Mirpur Mathelo, Ubauro. 24th September to 2nd October, 1929.

Post-epidemic period.

5th Survey—Ghotki, Mirpur Mathelo. 6th to 10th March, 1930.

6th Survey—Sukkur, Rohri, Pano Akil. 17th to 27th October, 1930.

GENERAL CHARACTERS OF THE DISTRICT.

Sukkur District is situated between $27^{\circ} 4'$ and $28^{\circ} 22'$ North latitude and $68^{\circ} 15'$ and $70^{\circ} 12'$ East longitude, and covers an area of approximately 5,600 square miles. It is very irregular in form, and is divided by the river Indus into two unequal portions. On the north it is bounded by the Upper Sind Frontier District, and by a detached portion of Bahawalpur State; on the east by Bahawalpur State and Jaisalmer State; on the south and south-west by Khairpur State; and on the west by Larkana and the Upper Sind Frontier Districts. The district is divided into eight talukas, of which three (Sukkur, Shikarpur and Garhi Yasin) are on the right bank of the Indus, and five (Rohri, Ghotki, Pano Akil, Mirpur Mathelo and Ubauro) are on the left.

A large part of the district, including the eastern portions of Rohri and Mirpur Mathelo talukas, consists of hillocks of wind-blown sand, running in parallel rows from north-east to south-west. This is known as the Registan, and is part of the great desert which continues southwards into Thar and Parkar District. The remainder of the district, with the exception of the hills south of Rohri, is a level plain covered with alluvial loam, and is very fertile.

There are no hills in the district, except the low range on the northern extremity of which Sukkur and Rohri are built, which runs southwards from that point to the Khairpur boundary. Its greatest elevation is about 480 feet above sea level, or about 300 above the surrounding country.

The Indus traverses the whole length of the district, and above Sukkur its course changes from year to year. At Sukkur it passes through the gorge between that town and Rohri, the island of Bukkur bisecting the channel. The Lloyd Barrage has been constructed at a point three miles below the gorge.

Formerly the district was subject to very extensive flooding from the Indus every year, but this is now to a great extent prevented by the construction of protective embankments. The tracts lying between the river and the embankments are subject to yearly inundations, and sometimes, as in 1929

* Only one village in each of these talukas was visited during this period.

and 1930, the embankments are breached by abnormal rises in the level of the river, and large areas may be submerged.

There is one other so-called river in the district, the Eastern Nara, which is sometimes spoken of as a natural branch of the Indus. It probably marks the course of the Hakro, or Lost River of Sind, which at some period abandoned its ancient bed and poured its flood into the Chenab or the Indus. Long after it ceased to be an independent river the Eastern Nara served as a channel by which the flood waters of the Indus were guided down to the Dhoru Puran and Kori Creek, and so into the Rann of Cutch. In 1859 it was converted into a canal by the opening of a controlled channel between it and the Indus at Rohri, and from it the Jamrao and Mithrao Canals, which irrigate large areas in Lower Sind, derive their supply. Under the Barrage Scheme the Eastern Nara receives a regular flow from the new Eastern Nara Channel, which comes off from the Indus immediately above the Barrage, and is the widest canal in the world. The bed of the Nara, for a distance of 236 miles, is being canalized, and its branch canals re-modelled or enlarged.

Climate.

The maximum and minimum temperatures recorded in Sukkur town for the years 1912 to 1921 are given in Table II. The climate generally speaking is the same as that which prevails throughout Northern Sind, the hot season lasting from the middle of March to the middle of October. Sukkur town, being situated on the banks of the river, is slightly cooler than the rest of the district.

Rainfall.

The average rainfall at Sukkur is from two to three inches annually, most of the rain falling in July and August. The rainfall figures for a number of years of the headquarter towns of the six talukas with which this paper is concerned are given in Table I

Population.

Approximately 70 per cent of the population are Mussulmans and 30 per cent Hindus, the proportion of the latter being higher than in any other district of Sind except Thar and Parkar and Hyderabad. Ghotki taluka contains the most densely populated rural tract (120 per square mile), whilst the desert taluka of Mirpur Mathelo has only 24 inhabitants to the square mile.

Cultivation.

Although the great canals which have been constructed under the Lloyd Barrage Scheme have their origin from the Indus just below Sukkur, no part of the district except portions of Garhi Yasin and Rohri talukas and small areas in Sukkur and Shikarpur talukas come under the operation of the Barrage Canals. A considerable portion of the district is, however, irrigated by inundation canals coming directly from the Indus at various points above the Barrage.

Of the summer crops, *juari* is the staple food crop of the district, and is grown in every taluka. *Bajri* is less extensively grown, but it is largely

cultivated in the sandy portions of Mirpur Mathelo taluka. Sesame is grown in every taluka, but not to any great extent. Rice is grown chiefly in Garhi Yasin taluka, though the area under this crop has been steadily increasing during recent years in Shikarpur and Sukkur talukas.

Of the winter crops, wheat is grown in every taluka, either in lands submerged by the spill of the river or of a canal (*sailabi* cultivation), or on lands which have been given a flooding by wheel irrigation towards the end of the inundation season (*bosi* cultivation). These methods are supplemented by winter irrigation by wheels erected on wells (*chahi* irrigation), or on the river or lakes (*dhako* irrigation). Other winter crops grown in various parts of the district are chickling vetch, gram, rape, jambho and tobacco. Fruit gardens are chiefly found at Shikarpur and Rohri, and there is a considerable amount of date palm cultivation at Rohri and Sukkur and along the banks of the Indus.

EPIDEMIC MALARIA.

The epidemic figures of the six talukas under review for the years 1901 to 1932 are given in Table III. During this period there have been three regional or fulminant epidemics, in the years 1906, 1917 and 1929. A reference to Table I will show that each of these years was characterized by exceptionally heavy monsoon rainfall. The causation of the regional epidemics which affect Northern Sind has been fully discussed in a previous paper (Covell and Baily, 1932). Briefly, it may be stated that these epidemics occur at intervals of approximately ten years, when the communal immunity to malaria has fallen to a very low level, and are precipitated by the sudden advent of conditions unusually favourable to the longevity of the malaria-carrying mosquito, and hence to the transmission of the disease, *i.e.*, extensive rainfall and flooding.

The various phases of the epidemic cycle may be defined as follows :—

The *pre-epidemic period* is that between the appearance of the immediate determining cause (*i.e.*, excessive monsoon rainfall), and the first increase of morbidity due to malaria.

The *epidemic period* is that between the first increase of morbidity due to malaria and its reversion to the normal level.

The *post-epidemic period* is that between the end of the epidemic proper and the time when the spleen rate has declined to its normal inter-epidemic status.

The *inter-epidemic period* follows the post-epidemic period, and continues till the beginning of the next pre-epidemic period.

These definitions are given here because the terms in question are frequently used in the following pages.

RESULTS OF THE SURVEYS.

1. SUKKUR TALUKA.

This taluka lies on the right bank of the river Indus, and has an area of 273 square miles, with a population of 135,627 (in 1931). The eastern portion of the taluka, near the river, is covered with forest. Irrigation is

carried on both from the Indus and from Government canals, the chief of which are the Sind and the Sukkur. About 95 per cent of the cultivation is by flow and the rest by lift. The results of spleen examinations carried out in this taluka are given in Table IV.

PUNJABI is a village with a population of about 500, situated 2 miles north-west of Sukkur, in the midst of dry cultivation. A canal distributary flows within a furlong of the village. The subsoil water level in October 1930 was 16 feet.

First survey (inter-epidemic).—The spleen rate in April 1927 was 20 per cent (25 observations).

Second survey (post-epidemic).—The spleen rate in October 1930 was 93 per cent (29 observations). The average enlarged spleen was 7.5 cm.*

RAHUJA is a village with a population of about 300, situated in the midst of dry crop cultivation 3 miles north of Sukkur. A canal distributary flows within a furlong of the village. The subsoil water level in October 1930 was 12 feet.

First survey (inter-epidemic).—The spleen rate in April 1927 was 4.5 per cent (22 observations).

Second survey (post-epidemic).—The spleen rate in October 1930 was 88 per cent (41 observations). The average enlarged spleen was 8.7 cm. The parasite rate was 60 per cent (20 observations, M. T., 11, B. T., 1). Crescents were observed in three cases.

ABAD LAKHA is a village with a population of about 1,000, situated in the midst of dry cultivation $4\frac{1}{2}$ miles north of Sukkur. There is a small amount of rice cultivation also about half a mile from the village. There are three large ponds, one in the centre of the village and two at its periphery. The subsoil water level in October 1930 was 14 feet.

First survey (inter-epidemic).—The spleen rate in April 1927 was *nil* (40 observations).

Second survey (post-epidemic).—The spleen rate in October 1930 was 85 per cent (104 observations). The average enlarged spleen was 8.0 cm. The parasite rate was 58 per cent (19 observations, M. T., 10, B. T., 1).

ARAIN is a village with a population of about 700, situated 6 miles north of Sukkur, in the midst of dry crop cultivation. There is also a small amount of rice cultivation about 400 yards from the village. The subsoil water level in October 1930 was 13 feet.

First survey (inter-epidemic).—The spleen rate in April 1927 was 8 per cent (62 observations).

Second survey (post-epidemic).—The spleen rate in October 1930 was 85 per cent (63 observations). The average enlarged spleen was 7.9 cm.

SHAHPUR is a village with a population of about 150, situated 4 miles north-west of Sukkur, in the midst of dry crop cultivation. There is also

* The measurements of the average enlarged spleen throughout this paper are given in terms of the distance in centimetres from the apex of the spleen to the umbilicus.

some rice cultivation about half a mile from the village. A small canal distributary flows within about 100 yards of the village. During 1930 the floods reached almost to the periphery of the village, and at the time of the second survey patches of water were still present, in some of which larvæ of *A. culicifacies* were collected. The subsoil water level in October 1930 was 11 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was nil (15 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 96 per cent (24 observations). The average enlarged spleen was 9.6 cm.

LAKHI is a village with a population of about 1,800, situated about 16 miles north of Sukkur, in the midst of dry cultivation. There is also a small area of rice cultivation about three-quarters of a mile from the village. In 1930 the floods extended to the periphery of the village, and at the time of the second survey there was still a large amount of stagnant water on three sides, but no anopheline larvæ were found in it. The subsoil water level in October 1930 was 15 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 4 per cent (103 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 93 per cent (150 observations). The average enlarged spleen was 7.3 cm. The parasite rate was 40 per cent (20 observations, M. T., 7, B. T., 1.)

DODOGOR is a village with a population of about 300, situated 10 miles to the north of Sukkur, in the midst of dry crop cultivation, but with some rice cultivation also within about 400 yards. Larvæ of *A. culicifacies* were collected from the bed of a canal distributary which runs within 100 yards of the village. The subsoil water level in October 1930 was 8 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 8 per cent (25 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 87 per cent (24 observations). The average enlarged spleen was 6.7 cm.

JAHAN KHAN is a village with a population of about 600, situated 8 miles north of Sukkur. The main cultivation of the area is dry crop, but the village itself is actually surrounded by a zone of rice cultivation. A small canal distributary flows within 100 yards of its periphery. Larvæ of *A. culicifacies* were collected from two small hollows containing water which had been drained from the ricefields in October 1930. The subsoil water level at that time was 12 feet. The floods of 1930 had reached almost to the edge of the village, and some water collections from this source still remained, but no larvæ were found in them.

First survey (inter-epidemic).—In April 1927 the spleen rate was 18 per cent (31 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 90 per cent (52 observations). The average enlarged spleen was 8.0 cm. The parasite rate was 30 per cent (10 observations, M. T., 2, B. T., 1.)

KASSIM is a village with a population of about 700, situated 9 miles north-east of Sukkur, in the midst of dry cultivation, but with a small patch

of rice cultivation within 400 yards of its periphery. Larvæ of *A. culicifacies* were collected from two borrow-pits close to the village in October 1930. The subsoil water level at that time was 13 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 5 per cent (106 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 83 per cent (60 observations). The average enlarged spleen was 9.0 cm.

RICHANJI is a village with a population of about 900, situated about 10 miles north-east of Sukkur. It is surrounded by date palm cultivation, and there is also some rice grown within 200 yards of the village. In October 1930 larvæ of *A. culicifacies* were collected from borrow-pits close to a small canal distributary which flows within 300 yards of the village. The subsoil water level was 13 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 28 per cent (128 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 87 per cent (60 observations). The average enlarged spleen was 8.0 cm.

GOSARJI is a village with a population of about 1,100, situated 10 miles north-west of Sukkur, in the midst of dry cultivation. There is also a little rice cultivation about a mile from the village. The subsoil water level in October 1930 was 14 feet. The floods in that year had reached the edge of the village.

First survey (inter-epidemic).—In April 1927 the spleen rate was nil (25 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 79 per cent (90 observations). The average enlarged spleen was 7.8 cm.

BAGARJI is a village with a population of about 1,700, situated 8 miles north-west of Sukkur, in the midst of dry crop cultivation, with a certain amount of rice cultivation also. Larvæ of *A. culicifacies* were collected in October 1930 from a collection of water which had been drained off from a ricefield. The subsoil water level was 14 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 5.7 per cent (87 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 76 per cent (121 observations). The average enlarged spleen was 8.4 cm.

OLD SUKKUR is a rural town with a population of about 4,800, situated on the bank of the river Indus. An inundation canal, the Sukkur Canal, takes off from the river immediately above the town, and skirts its northern boundary. There are a number of excavations and borrow-pits in and around the town, and larvæ of *A. culicifacies* were found in one of these in October 1930. The subsoil water level was 16 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 22 per cent (207 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 80 per cent (270 observations). The average enlarged spleen was 7.4 cm. The parasite rate was 30 per cent (40 observations, M. T., 11, B. T., 1.)

NEW SUKKUR is separated from Old Sukkur by the railway line. It is situated on the bank of the Indus, but even at low water levels before the completion of the Barrage, there were no pools left in the river bed. The Barrage Colony is situated in New Sukkur. In April 1927 the spleen rate was *nil* (90 observations). No observations were made here in 1930.

2. ROHRI TALUKA.

The taluka has an area of 1,629 square miles, with a population of 116,738 (in 1931). It is divided into two parts by the Eastern Nara. The eastern portion forms part of the Registan, or desert tract, and depends for its cultivation entirely on rain. The western portion is irrigated by canals, about 74 per cent of the cultivation being under flow. The cultivation is mainly dry crop, but in the neighbourhood of the river, there is a considerable amount of date palm cultivation, which entails heavy irrigation whilst the trees are young. The observations here recorded were confined to the tract lying to the west of the Eastern Nara. The results of spleen examinations made in this taluka are given in Table V.

ROHRI, the headquarter town of the taluka, has a population of about 11,100, and is situated on a rocky eminence on the left bank of the river Indus. Before the Lloyd Barrage Scheme came into operation (in 1932), the water supply of the Eastern Nara flowed along a channel taking off from the Indus immediately to the north of the town. Under the Barrage Scheme the Nara receives its supply from the new Eastern Nara Channel, which comes off just above the Barrage, *i.e.*, 3 miles below Rohri. The subsoil water level in October 1930 was 15 feet near the river, and from 20 to 25 feet on the opposite side of the town. The spleen rate was 68 per cent (160 observations), and the average enlarged spleen was 8.1 cm. No observations were carried out in Rohri prior to the epidemic of 1929.

THATLI and TANDO MOHAMED KHAN are two small villages lying close together 3 miles south-west of Rohri, in the midst of date palm groves, with a combined population of about 900. In October 1930 the subsoil water level was 14 feet, and the spleen rate was 81 per cent (63 observations). The average enlarged spleen was 7.5 cm. No observations were made in these villages prior to the epidemic of 1929.

BORAH is a village with a population of about 250, situated 3½ miles south-west of Rohri in the midst of date palm groves. In October 1930 the spleen rate was 88 per cent (35 observations). The average enlarged spleen was 7.7 cm. The subsoil water level was 14 feet. No observations were made in this village prior to the epidemic of 1929.

BEGMAJI is a village with a population of about 400, situated 6 miles south-west of Rohri, in the midst of dry crop cultivation. A canal distributary flows within 200 yards of the village. The subsoil water level in October 1930 was 15 feet. The spleen rate was 74 per cent (54 observations), and the average enlarged spleen was 9.7 cm. No observations were made in this village prior to the epidemic of 1929.

PATNI is a village with a population of about 900, situated 4 miles south-west of Rohri, in the midst of dry crop cultivation. A minor canal, the Umar

Khan Wah, flows within 100 yards of the village. The subsoil water level in October 1930 was 10 feet. The spleen rate was 88 per cent (62 observations), and the average enlarged spleen was 8.3 cm. No observations were made in this village prior to the epidemic of 1929.

KANDRA is a village with a population of about 1,300, situated 8 miles south-west of Rohri, in the midst of dry crop cultivation. The Umar Khan Wah is about three-quarters of a mile distant, and a distributary from this flows close to the village. The subsoil water level in October 1930 was 26 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 2 per cent (160 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 59 per cent (112 observations). The average enlarged spleen was 9.0 cm.

MIANI BHAGAT is a village with a population of about 200, situated one mile south-west of Rohri, in the midst of date palm groves, the remainder of the cultivation being dry crops. Larvæ of *A. culicifacies* were collected from the pits surrounding the young palm trees in October 1930. The subsoil water level was 12 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 38 per cent (26 observations).

Second survey (post-epidemic).—In October 1930 the spleen rate was 91 per cent (48 observations). The average enlarged spleen was 7.5 cm.

3. PANO AKIL TALUKA.

This taluka has an area of 393 square miles, with a population of 47,287 (in 1931). The portion which lies along the course of the Indus is low-lying and traversed by depressions caused by the vagaries of that river. Protective embankments have been made to prevent widespread flooding, and the country outside these, i.e., between the embankments and the river, is flooded annually during the inundation period. All the villages of Pano Akil taluka dealt with in this paper excepting Pano Akil itself and Nidhapur are outside the protective embankment, and thus are liable to annual flooding. The results of spleen examinations made in this taluka are given in Table VI.

PANO AKIL, the headquarters of the taluka, has a population of about 1,000, and is situated about 2 miles within the protective embankment, in the midst of dry crop cultivation and gardens. Larvæ of *A. culicifacies* were collected from some pits near a canal distributary which runs through the centre of the town. Larvæ of *A. stephensi* were found in a well. The subsoil water level was between 15 and 20 feet in October 1928, and between 10 and 12 feet in October 1930.

First survey (inter-epidemic).—In October 1928 the spleen rate was 12 per cent (120 observations), and the average enlarged spleen was 9.0 cm. The parasite rate was 4 per cent (75 observations, M. T., 2, B. T., 1.)

Second survey (post-epidemic).—In October 1930 the spleen rate was 67 per cent (70 observations), and the average enlarged spleen was 9.3 cm. The parasite rate was 42 per cent (40 observations, M. T., 17).

PITAFI is a village with a population of about 200, situated 3 miles north-west of Pano Akil, outside the protective embankment. In October 1928

larvæ of *A. culicifacies* were collected from the village pond. The subsoil water level was 14 feet. The spleen rate was 10 per cent (20 observations), and the parasite rate 21 per cent (19 observations, M. T., 1, B. T., 3). No observations were made in this village in 1930, because the inhabitants had all left it on account of the floods.

URL and JANI JO are two small villages with a combined population of about 450, situated close together 3 miles north-west of Pano Akil, outside the protective embankment. The subsoil water in October 1928 was 15 feet.

First survey (inter-epidemic).—In October 1928 the spleen rate was 28 per cent (61 observations), and the average enlarged spleen was 9.4 cm. The parasite rate was 23 per cent (61 observations, M. T., 8, B. T., 7).

Second survey (post-epidemic).—In October 1930 the spleen rate was 90 per cent (41 observations), and the average enlarged spleen was 8.0 cm.

SHAHPUR is a village with a population of about 150, situated 7 miles north-west of Pano Akil, outside the protective embankment. The subsoil water level is said to vary with the rise and fall of the Indus. In October 1928 it was 11 feet.

First survey (inter-epidemic).—In October 1928 the spleen rate was 27 per cent (44 observations), and the average enlarged spleen was 9.0 cm. The parasite rate was 21 per cent (43 observations, M. T., 7, B. T., 2).

Second survey (post-epidemic).—In October 1930 the spleen rate was 70 per cent (10 observations only), and the average enlarged spleen was 6.4 cm.

SADHUJA is a village with a population of about 300, situated 8 miles north-west of Pano Akil, outside the protective embankment. The subsoil water level, which is said to vary with the rise and fall of the river, was 13 feet in both October 1928 and October 1930.

First survey (inter-epidemic).—In October 1928 the spleen rate was 25 per cent (36 observations), and the average enlarged spleen was 8.7 cm. The parasite rate was 8 per cent (35 observations, M. T., 1, B. T., 3).

Second survey (post-epidemic).—In October 1930 the spleen rate was 91 per cent (75 observations), and the average enlarged spleen was 8.4 cm.

SARAI KALWAR is a village with a population of about 400, situated 6 miles north of Pano Akil, just inside the flood restriction embankment. The subsoil water level in both October 1928 and October 1930 was 13 feet.

First survey (inter-epidemic).—In October 1928 the spleen rate was 25 per cent (52 observations), and the average enlarged spleen was 8.7 cm. The parasite rate was 12 per cent (50 observations, M. T., 3, B. T., 3).

Second survey (post-epidemic).—In October 1930 the spleen rate was 68 per cent (28 observations), and the average enlarged spleen was 10.3 cm.

NIDHAPUR is a village with a population of about 400, situated just inside the flood restriction embankment 3 miles north of Pano Akil. The subsoil water level in October 1928 was 12 feet.

First survey (inter-epidemic).—In October 1928 the spleen rate was 28 per cent (110 observations), and the average enlarged spleen was 9.9 cm. The parasite rate was 28 per cent (30 observations, M. T., 5, B. T., 2).

Second survey (post-epidemic).—In October 1930 the spleen rate was 62 per cent (40 observations), and the average enlarged spleen was 9·8 cm.

FATEH KHAN JO GOT, SATARNA, KHAN BELA and SAMO CHACHAR are four small villages situated near one another outside the flood restriction embankment, 5 miles north-west of Pano Akil. The results of observations made in October 1928 on the few children available in these villages are given in Table VI. No observations were possible in October 1930, as the inhabitants had left their homes on account of the floods.

4. GHOTKI TALUKA.

This taluka has an area of 345 square miles and a population of 43,978 (in 1931). It is a narrow tract of country, situated along the course of the Indus. The general level of the ground is low, and the whole taluka is traversed by depressions caused by the vagaries of the river. It is partly watered by the Lundi, Mahesro and Dengro canals. The land outside the flood restriction embankment, which is flooded by the rise of the river in the inundation season, produces luxuriant wheat crops. Eighty-three per cent of the cultivation is under flow. The results of spleen examinations made in this taluka are given in Table VII.

GHOTKI, the headquarter town of the taluka, has a population of about 3,700, and is situated about 4 miles south of the Indus in the midst of dry crop cultivation. A flood protection embankment has been constructed about 2 miles north of the town. The sub-soil water level was 3 feet in September 1929, and 20 feet in March 1930.

First survey (inter-epidemic).—In January 1928 the spleen rate was 7 per cent (85 observations).

Second survey (early epidemic).—In September 1929 the spleen rate was 5 per cent (239 observations), and the average enlarged spleen was 8·9 cm. The parasite rate was 64 per cent (50 observations, M. T., 31, B. T., 1).

Third survey (early post-epidemic).—In March 1930 the spleen rate was 25 per cent (200 observations) and the average enlarged spleen was 7·7 cm. The parasite rate was 38 per cent (50 observations, M. T., 13, B. T., 6).

QADERPUR is a village with a population of about 1,700, situated 5 miles north-west of Ghotki in the midst of dry crop cultivation. Extensive flooding occurred in this area in 1929. The subsoil water level in September 1929 was 3 feet, and in March 1930 it was 10 feet.

First survey (early epidemic).—In September 1929 the spleen rate was 7 per cent (145 observations), and the average enlarged spleen was 7·7 cm. The parasite rate was 76 per cent (50 observations, M. T., 34, B. T., 5). Crescents were observed in one case.

Second survey (early post-epidemic).—In March 1930 the spleen rate was 46 per cent (134 observations) and the average enlarged spleen was 7·0 cm. The parasite rate was 66 per cent (50 observations, M. T., 29, B. T., 4). Crescents were observed in 1 case.

HUSSAIN BELI is a village with a population of about 400, situated 1½ miles west of Ghotki in the midst of dry crop cultivation. In 1929 the floods extended to within a mile of this village. The subsoil water level in March 1930 was 19 feet.

First survey (early epidemic).—In September 1929 the spleen rate was 12 per cent (75 observations), and the average enlarged spleen was 9.6 cm. The parasite rate was 85 per cent (48 observations, M. T., 34, B. T., 8). Crescents were observed in one case.

Second survey (early post-epidemic).—In March 1930 the spleen rate was 47 per cent (47 observations) and the average enlarged spleen was 8.7 cm. The parasite rate was 60 per cent (35 observations, M. T., 18, B. T., 3). Crescents were observed in 6 cases.

MATHELO is a village with a population of about 500, situated 2 miles south-east of Ghotki, in an old fort. There is dry crop cultivation extending to within 200 yards of the village. The subsoil water level in September 1929 was 20 feet.

First survey (early epidemic).—In September 1929 the spleen rate was 11 per cent (85 observations), and the average enlarged spleen was 9.0 cm. The parasite rate was 68 per cent (50 observations, M. T., 30, B. T., 3, Q., 1). Crescents were observed in 2 cases.

Second survey (early post-epidemic).—In March 1930 the spleen rate was 50 per cent (20 observations), and the average enlarged spleen was 9.2 cm.

5. MIRPUR MATHELO TALUKA.

This taluka has an area of 1,604 square miles, with a population of 56,656 (in 1931). A large part of the taluka consists of sandhills and desert. The country in former years was traversed by the Bahawalpur and Raharki floods, which came to it through Ubauro taluka, but since they have been checked by protective embankments, the Mahi Wah system of canals has been constructed and this irrigates the western portion of the taluka. Sixty-one per cent of the cultivation is under flow. Owing to the widespread floods which occurred in 1929 it was not possible to make an extensive survey in this taluka, and observations were only made in one locality. The results of spleen examinations made in this taluka are given in Table VIII.

MIRPUR MATHELO, the headquarters of the taluka, has a population of about 1,800, and is situated in the midst of dry crop cultivation about 8 miles south of the Indus. A canal, the Maso Wah, flows close by the town. The subsoil water level in March 1930 was 20 feet.

First survey (early epidemic).—The spleen rate was 18 per cent (135 observations), and the average enlarged spleen was 8.7 cm. The parasite rate was 81 per cent (83 observations, M. T., 59, B. T., 8). Crescents were observed in 10 cases.

Second survey (early post-epidemic).—The spleen rate was 61 per cent (85 observations), and the average enlarged spleen was 7.0 cm. The parasite rate was 70 per cent (50 observations, M. T., 33, B. T., 2). Crescents were observed in 4 cases.

6. UBAURO TALUKA.

This taluka has an area of 462 square miles, with a population of 46,146 (in 1931). Like Mirpur Mathelo, it was formerly fertilized by the two floods, but is now under irrigation from canals, the chief of these being the Mahi, Dahar, Maharo and Sehar. Only 35 per cent of the cultivation is under flow. The results of spleen examination made in this taluka are given in Table IX.

UBAURO, the headquarters of the taluka, has a population of about 1,700, and is situated on the banks of the Mahi Wah, about 10 miles south-east of the Indus. It is protected from floods by an embankment. The subsoil water level in September 1929 was 12 feet.

Spleen and blood results (early epidemic).—In September 1929 the spleen rate was 10 per cent (144 observations), and the average enlarged spleen was 9.3 cm. The parasite rate was 86 per cent (50 observations, M. T., 42, B. T., 1). Crescents were observed in 4 cases.

JHANGAL MOHANA is a small village with a population of about 50, situated $1\frac{1}{2}$ miles north of Ubauro, on the banks of the Mahi Wah. The spleen rate in September 1929 (early epidemic period) was 17 per cent (12 observations only).

LANGHA is a village with about 300 inhabitants, situated 4 miles north of Ubauro, close to the Mahi Wah. The spleen rate in September 1929 (early epidemic period) was 28 per cent (53 observations), and the average enlarged spleen was 8.0 cm.

MATAR KOT is a village with a population of about 200, situated 3 miles east of Ubauro, in the midst of dry cultivation. The spleen rate in September 1929 (early epidemic period) was 16 per cent (32 observations), and the average enlarged spleen was 8.6 cm.

DHARKI is a village with about 1,600 inhabitants, situated 6 miles south of Ubauro, in the midst of dry crop cultivation. There is a large pond at the periphery of the village. In September 1929 the subsoil water level was 22 feet.

Spleen and blood results (early epidemic).—In September 1929 the spleen rate was 14 per cent (149 observations), and the average enlarged spleen was 8.2 cm. The parasite rate was 72 per cent (50 observations, M. T., 33, B. T., 3). There were 3 crescent carriers.

DISSECTIONS OF ANOPHELINE MOSQUITOES.

During the week commencing 24th September, 1929, 74 specimens of *A. culicifacies*, caught in villages in Ghotki, Mirpur Mathelo and Ubauro, were dissected. Of these, 7 (9.5 per cent) showed gut infections, and 4 (5.4 per cent) showed gland infections, the total infection rate being 13.5 per cent. These figures were included in our paper dealing with the regional epidemic in Northern Sind (Covell and Baily, 1932). Eighty per cent of the anophelines caught at this period were *A. culicifacies*.

DISCUSSION OF RESULTS.**ENDEMIC MALARIA.**

The results of our observations throughout Upper Sind have shown, in every locality visited since 1929, the profound effect of the epidemic which occurred in that year. Surveys carried out from 8 to 10 months after the end of the epidemic period have shown that the spleen rate was everywhere raised to a very high figure, usually in the neighbourhood of 80 to 90 per cent. Observations made prior to the epidemic, and in the very early stages of the epidemic itself, however, show that the amount of endemic malaria varied considerably in different localities, depending on the presence or absence of breeding places favourable to *A. culicifacies* under normal conditions.

The localities under review in the present paper may conveniently be considered under the following headings :—

- (1) Villages in areas of dry crop cultivation.
- (2) Villages surrounded by date palm groves.
- (3) Villages lying outside the flood restriction embankments, *i.e.*, between these and the river Indus.

Villages in areas of dry crop cultivation.

The results of spleen examinations in these villages are shown in Table X. The spleen rates recorded prior to the epidemic of 1929 were for the most part very low, usually between 5 and 10 per cent. In the few cases where the figures were slightly higher, the increase could be attributed to the presence of a certain amount of rice cultivation, or to the immediate proximity of an inundation canal. Old Sukkur gave a figure of 22 per cent, the comparatively high incidence of malaria in this case being probably due to the breeding of *A. culicifacies* along the bed of the Indus. Larvæ of this species were collected from pools in the river bed and at the head of the Sukkur Canal in April 1927. Spleen rates recorded during the early stages of the epidemic, when the rate had already begun to rise, were still low, being mostly between 10 and 15 per cent.

Villages in the midst of date palm groves.

As has been noted above, this type of cultivation entails heavy irrigation, especially during the early years of growth of the trees, and the water in the pits dug round them forms favourable breeding places for *A. culicifacies*. Spleen rates recorded in two of these villages are given in Table XI, these being the only villages with this type of cultivation in which observations were made in Sukkur District prior to the epidemic. The spleen rates (28 per cent and 38 per cent respectively) are considerably higher than those in the dry crop area.

Villages lying between the Indus and the flood restriction embankments.

The spleen rates recorded in this tract prior to the epidemic are given in Table XII. The area, as already explained, is subject to flooding every year in the inundation season. The amount of endemic malaria in the tract is considerable, the spleen rates for the most part being between 25 and 30 per cent.

The high incidence of malaria in this tract was also commented on by Young and Majid (1930), who recorded spleen rates in the neighbourhood of 60 per cent in two villages (Dubar and Mundodero), in Rohri taluka, lying outside the protection of the flood restriction embankment.

THE REGIONAL EPIDEMIC OF 1929.

The results of an intensive study of the regional epidemic in Northern Sind, based on observations made in Shikarpur and the neighbouring villages, have already been published (Covell and Baily, 1932). The data recorded in the present paper tend to corroborate the findings arrived at in Shikarpur taluka in regard to the main features of the epidemic. The results of observations made at various periods are summarized in Tables XIII, XIV and XV.

The only observations made in the area now under review during the epidemic period were carried out during the week commencing 24th September, 1929, i.e., approximately 4 weeks after its commencement. The principal features to be noted are the high parasite rate (76 per cent), contrasted with the low spleen rate (11 per cent). The average enlarged spleen was 8.7 cm. The M. T. parasite rate was 69 per cent, and the B. T. rate 7 per cent.

Observations made in the same localities in the period 6th to 10th March, 1930, i.e., about 2½ months after the end of the epidemic gave the following results:—Spleen rate 41 per cent, average enlarged spleen 7.5 cm. parasite rate 58 per cent (M. T., 50 per cent, B. T., 8 per cent). It will be seen that in comparison with the September figures there had been a marked increase in the spleen rate, together with an increase in the size of the average enlarged spleen. As regards the parasite rate, the percentage of M. T. infections had fallen from 69 to 50, whilst the B. T. infection rate remained at approximately the same low figure as in the early stages of the epidemic period. The intensity of infections in both cases showed a very marked fall.

These findings are in accord with those recorded by us in Shikarpur (Covell and Baily, 1932). For reasons which were fully discussed in that paper, we considered that the increase in the spleen rate together with the increase in the size of the average enlarged spleen which occurred during the latter part of the epidemic period were due chiefly to early relapses of M. T. infections.

One of the most striking features brought out in our studies of the regional epidemic of 1929 has been the very marked increase in the spleen rate during the first year after the epidemic period, associated with a decrease in the size of the average enlarged spleen. In the case of Shikarpur, observations made in August 1930 showed that in this area there had been a considerable increase in the number of B. T. infections since January 1930, whilst the M. T. infection rate had fallen to a very low figure. We considered that these findings could only be explained by the occurrence, during the spring and early summer, of delayed primary attacks or relapses of malaria resulting from *P. vivax* infections acquired during the previous autumn.

In the present instance, no observations were made between March and October 1930. Those made in the latter month were confined to the talukas of Sukkur, Rohri and Pano Akil, which had not been visited since before the commencement of the epidemic. The spleen rate was now 80 per cent, the

average enlarged spleen 80 cm., and the parasite rate 42 per cent (M. T., 40 per cent., B. T., 2 per cent). From what we know of the course of events in Shikarpur, it seems possible that the marked rise of the spleen rate associated with a decrease in the size of the average enlarged spleen may be attributable in this case also to delayed primary attacks or relapses of B. T. infections which had occurred in the early part of the year, although there is no direct evidence to this effect. The comparatively high M. T. infection rate is what one would expect, since the observations were made after the commencement of the autumnal malaria season of 1930.

THE PROBABLE EFFECT OF THE LLOYD BARRAGE SCHEME ON MALARIA IN SUKKUR DISTRICT.

The only part of Sukkur District which will be irrigated directly from the Barrage Canals is the southern portion of Garhi Yasin taluka and small areas in Rohri, Sukkur and Shikarpur talukas. The three great main canals on the right bank of the Indus, however, pass through Sukkur taluka in the first part of their course. Similarly, the four main left bank canals run through the northern portion of Rohri taluka. Should there be any leakage from the canals, or any considerable rise in the subsoil water level, an increase in the incidence of malaria may be expected in the affected area.

There has been recently a considerable rise in the subsoil water level in Shikarpur taluka, and certain areas have been threatened with water-logging. This has been attributed not to the operation of the Barrage Scheme, but to the great increase in rice cultivation which has taken place since 1924, and to some extent to the relatively heavy rainfall and floods of 1929 and 1930. It is understood that the situation is to be met by restricting the area under rice cultivation and by the installation of drainage which will presumably be linked with the main drainage system of the right bank area of the Barrage Command. A continuance of the present conditions will certainly lead to an increase of malaria in this area.

On the left bank of the Indus there is an area of about 17 square miles lying between the Aror Hills and the new Eastern Nara Channel, which has given very definite signs of being water-logged. This area, like that in Shikarpur taluka, is under investigation by the Irrigation Department.

SUMMARY.

1. An account is given of malaria surveys carried out in various localities in Sukkur District (a) prior to the regional epidemic of 1929, (b) in the 4th week of the epidemic, (c) 2½ months after the epidemic, and (d) 10 months after the epidemic.

2. Dry crop cultivation is carried out over most of the district, and the amount of endemic malaria is generally low, except in tracts lying between the Indus and the flood restriction embankments, which are subject to annual inundations. The incidence of endemic malaria is also increased in villages situated in the midst of date palm cultivation, the method of irrigating the young trees affording favourable breeding places for *A. culicifacies*.

3. The main features of the epidemic of 1929 were similar to those recorded in Shikarpur and the neighbouring villages (Covell and Baily, 1932), and in the Upper Sind Frontier District (Covell and Baily, 1931).

4. The possible effects of the operation of the Lloyd Barrage Scheme on the incidence of malaria in Sukkur District are discussed.

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APPENDIX.

TABLE I.

Rainfall figures recorded at the headquarters of six talukas of Sukkur District during the months July to September, 1901 to 1933.

Years	SUKKUR.			ROHEL.			PANO AKIL.			GHOTKI.			MIRPUR MATHELO.			UBAURO.			REMARKS.
	July.	August.	September.	July.	August.	September.	July.	August.	September.	July.	August.	September.	July.	August.	September.	August.	September.		
1901 ..	0.80	1.10	0.45	0.92	0.02	..	0.08	September.	
1902	0.38	0.43	..	0.06	0.22	0.65	0.07	..	0.48	0.03	..	2.69	August.	
1903 ..	2.49	2.76	..	0.02	..	1.93	1.43	0.05	..	2.96	
1904	
1905	
1906	3.44	2.80	2.67	0.52	..	3.89	2.73	
1907 ..	0.05	1.05	..	0.08	0.99	1.24	1.96	3.44	
1908 ..	0.71	1.20	..	0.72	1.00	0.51	0.84	..	2.04	1.48	..	3.36	0.10	
1909 ..	0.87	1.31	1.60	0.91	1.08	
1910 ..	0.76	0.04	..	0.83	0.02	..	5.50	2.60	..	1.68	1.12	0.23	..	1.13	0.38	
1911 ..	0.18	5.50	3.60	0.20	0.58	
1912 ..	1.52	2.60	..	1.83	3.00	..	0.30	2.42	..	1.24	0.24	2.16	..	0.85	0.62	
1913 ..	0.99	0.44	0.06	1.48	0.87	..	5.50	1.51	1.35	0.20	0.23	3.35	0.04	0.42	3.27	
1914 ..	2.26	2.39	2.23	..	0.85	0.89	2.98	..	0.20	5.22	0.02	0.04	

[illegible]

TABLE II.
Temperatures recorded at Sukkur town, 1912 to 1921.

Year.	JANUARY.		MAY.		JULY.		NOVEMBER.	
	Max. °F.	Min. °F.	Max. °F.	Min. °F.	Max. °F.	Min. °F.	Max. °F.	Min. °F.
1912 ..	77	60	112	90	117	90	93	60
1913 ..	82	60	117	88	115	87	104	67
1914 ..	85	60	121	92	117	87	96	72
1915 ..	84	56	118	86	116	88	98	66
1916 ..	82	60	113	82	110	88	94	66
1917 ..	82	60	108	82	113	90	96	64
1918 ..	82	52	116	90	114	85	96	68
1919 ..	84	54	113	84	116	86	92	68
1920 ..	76	60	94	80	108	84	88	67
1921 ..	76	62	110	85	112	76	80	49

TABLE III.
Epidemic figures of six talukas of Sukkur District during the years 1901 to 1932.

Year.	Sukkur.	Rohri.	Pano Akil.	Ghotki.	Mirpur Mathelo.	Ubauro.	REMARKS.
1901 ..	2.62	3.35	..	1.59	1.40	1.48	Epidemic.
1902 ..	1.80	2.09	..	1.52	2.26	1.14	
1903 ..	2.54	2.68	..	1.63	2.86	2.63	
1904 ..	2.12	1.65	..	1.36	1.48	1.75	
1905 ..	2.44	2.65	2.55	2.48	3.31	2.53	
1906 ..	8.77	8.84	10.37	20.04	19.66	13.78	
1907 ..	2.91	2.36	..	3.39	3.97	2.75	
1908 ..	2.86	2.13	3.55	5.82	3.13	2.53	
1909 ..	1.44	2.11	1.75	2.44	1.64	1.75	
1910 ..	2.07	2.55	1.02	3.11	2.17	2.78	
1911 ..	1.21	1.46	1.15	1.90	0.48	1.58	
1912 ..	1.27	0.44	1.27	1.41	2.46	1.21	
1913 ..	2.90	3.03	1.92	2.46	3.31	1.12	
1914 ..	2.68	1.53	2.01	1.62	2.02	1.65	
1915 ..	3.61	1.28	1.57	1.47	1.28	1.38	Epidemic. Influenza.
1916 ..	3.20	2.17	3.55	3.04	4.44	2.56	
1917 ..	8.98	20.48	9.05	9.17	6.08	15.02	
1918	
1919	
1920 ..	1.22	0.67	1.75	0.74	1.04	1.17	
1921 ..	1.02	1.01	1.42	1.45	1.44	1.14	
1922 ..	2.45	1.07	1.03	1.04	0.88	1.12	
1923 ..	2.07	1.09	2.15	2.02	1.15	1.14	
1924 ..	2.47	1.01	5.87	4.27	2.11	2.82	
1925 ..	1.37	0.59	2.62	1.43	1.73	1.48	
1926 ..	1.89	1.51	3.06	2.30	2.04	1.12	
1927 ..	1.15	0.78	1.45	1.32	2.08	1.07	Epidemic.
1928 ..	1.06	0.93	1.42	1.03	1.01	1.43	
1929 ..	9.04	7.06	3.55	3.65	4.15	6.05	
1930 ..	2.54	1.03	1.60	1.60	0.71	1.20	
1931 ..	1.27	0.88	1.60	1.60	1.04	1.80	
1932 ..	2.56	2.20	2.10	2.10	2.30	1.50	

TABLE IV.
Results of spleen examinations, Sukkur taluka.

Locality.	INTER-EPIDEMIC PERIOD.			POST-EPIDEMIC PERIOD.			
	Date.	Number examined.	Spleen rate.	Date.	Number examined.	Spleen rate.	Average enlarged spleen.*
Punjabi ..	iv. 27	25	20.0	x. 30	29	93.0	7.5
Rahuja ..	iv. 27	22	4.5	x. 30	41	88.0	8.7
Abad Lakha ..	iv. 27	40	0.0	x. 30	104	85.6	8.0
Arain ..	iv. 27	62	8.0	x. 30	63	85.7	7.9
Shahpur ..	iv. 27	15	0.0	x. 30	24	95.8	9.6
Lakhi ..	iv. 27	103	3.9	x. 30	150	92.7	7.3
Dodogot ..	iv. 27	25	8.0	x. 30	24	87.5	6.7
Jahan Khan ..	iv. 27	31	17.9	x. 30	52	90.4	8.0
Kassim ..	iv. 27	106	4.7	x. 30	60	83.3	9.0
Bichanji ..	iv. 27	128	28.1	x. 30	60	86.7	8.0
Gosarji ..	iv. 27	25	0.0	x. 30	90	78.9	7.8
Bagarji ..	iv. 27	87	5.7	x. 30	121	76.0	8.4
Old Sukkur ..	iv. 27	207	22.2	x. 30	270	80.4	7.4
New Sukkur ..	iv. 27	90	0.0
TOTAL ..	iv. 27	966	11.8	x. 30	1,088	84.5	..

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE V.
Results of spleen examinations, Rohri taluka.

Locality.	INTER-EPIDEMIC PERIOD.			POST-EPIDEMIC PERIOD.			
	Date.	Number examined.	Spleen rate.	Date.	Number examined.	Spleen rate.	Average enlarged spleen.*
Kandra ..	iv. 27	160	2.5	x. 30	112	59.0	9.0
Miani Bhagat ..	iv. 27	26	38.5	x. 30	48	91.5	7.5
Thatli and Tando Mohd. Khan.	x. 30	63	81.0	7.7
Borah	x. 30	35	88.6	7.7
Begmaji	x. 30	54	74.0	9.7
Patni	x. 30	62	88.7	8.3
Rohri	x. 30	160	68.0	8.1
TOTAL ..	iv. 27	186	8.0	x. 30	534	73.8	..

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE VI.
Results of spleen examinations, Pano Akil taluka.

Locality.	INTER-EPIDEMIC PERIOD.				POST-EPIDEMIC PERIOD.			
	Date.	Number examined.	Spleen rate.	Average enlarged spleen.*	Date.	Number examined	Spleen rate.	Average enlarged spleen.*
Pano Akil ..	x. 28	120	12.5	9.0	x. 30	70	67.0	9.3
Url and Jani Jo.	x. 28	61	27.9	9.4	x. 30	41	90.2	8.0
Shahpur ..	x. 28	41	27.3	9.0	x. 30	10	70.0	6.4
Sadhuja ..	x. 28	36	25.0	8.7	x. 30	75	91.0	8.4
Sarai Kalwar	x. 28	52	25.0	8.7	x. 30	28	68.0	10.3
Nidhapur † ..	x. 28	110	28.2	9.9	x. 30	40	62.5	9.8
Pitafi ..	x. 28	20	10.0	..	x. 30	21	57.0	7.0
Fateh Khan Jo Got.	x. 28	20	15.0
Satarna ..	x. 28	21	28.6
Khan Bela ..	x. 28	10	20.0
Samo Chachar	x. 28	24	8.3
TOTAL ..	x. 28	518	22.2	..	x. 30	285	75.0	..

* Measurement in centimetres from apex of spleen to umbilicus.

† The spleen rate in this village in January 1928 was 62 per cent (74 observations).

TABLE VII.
Results of spleen examinations, Ghotki taluka.

Locality.	EARLY EPIDEMIC PERIOD.				EARLY POST-EPIDEMIC PERIOD.			
	Date.	Number examined.	Spleen rate.	Average enlarged spleen.*	Date.	Number examined.	Spleen rate.	Average enlarged spleen.*
Ghotki † ..	ix. 29	239	4.6	8.9	iii. 30	200	25.5	7.7
Qaderpur ..	ix. 29	145	6.9	7.7	iii. 30	134	46.2	7.0
Hussain Beli	ix. 29	75	12.0	9.6	iii. 30	47	46.8	8.7
Mathelo ..	ix. 29	85	10.7	9.0	iii. 30	20	50.0	9.2
TOTAL ..	ix. 29	544	7.3	..	iii. 30	401	36.0	..

TABLE VIII.
Results of spleen examinations, Mirpur Mathelo taluka.

Mirpur Mathelo	x. 29	135	18.0	8.7	iii. 30	85	61.0	7.0
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* Measurement in centimetres from apex of spleen to umbilicus.

† The spleen rate in Ghotki in January 1928 was 7 per cent (85 observations).

TABLE IX.
Results of spleen examinations, Ubauro taluka.

Locality.	EARLY EPIDEMIC PERIOD.			
	Date.	Number examined.	Spleen rate.	*Average enlarged spleen.*
Ubauro ..	ix. 29	144	9.7	9.3
Jhangal Mohana ..	ix. 29	12	16.6	..
Langha ..	ix. 29	53	28.3	8.0
Matar Kot ..	ix. 29	32	15.6	8.6
Dharki ..	ix. 29	140	14.1	8.2
TOTAL ..	ix. 29	390	14.6	8.6

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE X.

Spleen rates recorded in dry crop areas prior to the epidemic of 1929, or in its early stages.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.
INTER-EPIDEMIC PERIOD.				
Punjabi	iv. 27	25	5	20.0
Rahuja ..	iv. 27	22	1	4.5
Abad Lakha	iv. 27	40	0	0.0
Arain ..	iv. 27	62	5	8.0
Shahpur	iv. 27	15	0	0.0
Lakhi ..	iv. 27	103	4	3.9
Dodogot	iv. 27	25	2	8.0
Jahan Khan	iv. 27	31	5	17.9
Kassim ..	iv. 27	106	5	4.7
Gosarji ..	iv. 27	25	0	0.0
Bagarji ..	iv. 27	87	4	5.7
Old Sukkur	iv. 27	207	47	22.2
New Sukkur	iv. 27	90	0	0.0
Pano Akil	x. 28	120	15	12.5
Pitafi ..	x. 28	20	2	10.0
Kandra	iv. 27	160	4	2.5
EARLY EPIDEMIC PERIOD.				
Ghotki ..	ix. 29	239	11	4.6
Qaderpur ..	ix. 29	145	10	6.9
Hussain Beli	ix. 29	75	9	12.0
Mathelo ..	ix. 29	85	9	10.7
Mirpur Mathelo	x. 29	139	24	18.0
Ubauro ..	ix. 29	144	14	9.7
Jhangal Mohana	ix. 29	12	2	16.6
Langha ..	ix. 29	53	15	28.3
Matar Kot	ix. 29	32	5	15.6
Dharki ..	ix. 29	149	21	14.1

TABLE XI.

Spleen rates recorded in villages surrounded by date palm groves, prior to the epidemic of 1929.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.
INTER-EPIDEMIC PERIOD.				
Miani Bhagat ..	iv. 27	26	10	38·5
Bichanji ..	iv. 27	128	36	28·1

TABLE XII.

Spleen rates recorded in the tract between the Indus and the flood restriction embankments, prior to the epidemic of 1929.

[INTER-EPIDEMIC PERIOD.				
Url and Jani Jo ..	x. 28	61	17	27·9
Shahpur ..	x. 28	44	12	27·3
Sadhuja ..	x. 28	36	9	25·0
Sarai Kalwar* ..	x. 28	52	13	25·0
Nidhapur* ..	x. 28	110	31	28·2
Fateh Khan Jo Got ..	x. 28	20	3	15·0
Satarna ..	x. 28	21	6	28·6
Khan Bela ..	x. 28	10	2	20·0
Samo Chachar ..	x. 28	24	2	8·3

* These two villages are actually situated just within the protecting embankment, but the flood waters from the Indus reach to within a few yards of their periphery every year. The spleen rate at Nidhapur in January 1928 was 62 per cent (74 observations).

TABLE XIII.

Spleen rates recorded at different periods in Sukkur District, 1927 to 1930.

Name of taluka.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
INTER-EPIDEMIC PERIOD.					
Sukkur, Rohri	iv. 27	1,152	128	11·0	..
Pano Akil	x. 28	518	112	21·6	8·6
EARLY EPIDEMIC PERIOD.					
Ghotki, Mirpur Mathelo, Ubauro	ix. 29	1,069	121	11·3	8·7
EARLY POST-EPIDEMIC PERIOD.					
Ghotki, Mirpur Mathelo ..	iii. 30	486	198	40·7	7·5
LATER POST-EPIDEMIC PERIOD.					
Sukkur, Rohri, Pano Akil ..	x. 30	1,907	1,528	80·1	8·1

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE XIV.

Results of blood examinations made at different periods in Sukkur District, 1928 to 1930.

Name of taluka.	Date.	Number examined.	Number with parasites.	Parasite rate.	M. T. infection rate.	B. T. infection rate.	Q. infection rate.
INTER-EPIDEMIC PERIOD.							
Pano Akil ..	x. 28	394	72	18	13.2	5.6	0.0
EARLY EPIDEMIC PERIOD.							
Ghotki, Mirpur Mathelo, Ubauro.	ix. 29	381	291	76	69.0	8.1	0.5
EARLY POST-EPIDEMIC PERIOD.							
Ghotki, Mirpur Mathelo	iii. 30	185	108	58	50.2	8.1	0.0
LATER POST-EPIDEMIC PERIOD.							
Sukkur, Rohri, Pano Akil	x. 30	149	63	42	39.6	3.4	0.0

TABLE XV.

Results of parasite counts recorded in Sukkur District at different periods, 1928 to 1930.

Number of parasites per c.mm. of blood.	OCTOBER 1928.		SEPTEMBER 1929.		MARCH 1930.		OCTOBER 1930.	
	M. T.	B. T.	M. T.	B. T.	M. T.	B. T.	M. T.	B. T.
1-100 ..	3	2	1	1	21	3	7	0
100-500 ..	30	15	63	5	46	7	35	4
500-1,000 ..	11	1	44	1	14	3	8	0
1,000-5,000 ..	5	2	121	9	8	2	5	0
5,000-10,000 ..	0	0	20	3	4	0	2	0
over 10,000 ..	0	0	10	8	0	0	1	0
TOTAL ..	49	20	259	27	93	15	58	4

Note.—Mixed infections have been omitted. There was one quartan infection noted in September 1929, the count being between 1,000 and 5,000 per c.mm.

MALARIA IN SIND.

Part X.

MALARIA IN DADU DISTRICT.

BY

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[30th January, 1934.]

INTRODUCTION.

PERIOD AND SCOPE OF SURVEYS.

THE investigations on which the present paper is based were carried out during five visits to the district, which were made during the period 1928-1932. A severe regional epidemic of malaria, with its centre in Upper Sind, occurred in the autumn of 1929, and this affected Dadu District to a considerable extent, though its results were not nearly so serious as in the case of the more northerly situated districts of the province. The first three surveys were made prior to the commencement of the epidemic, and the remaining two during the post-epidemic period. The names of the talukas visited, and the periods at which the surveys were made, are given below :—

Inter-epidemic period.

1st survey—Kakar. 2nd January, 1928.

2nd survey—Sehwan. 22nd August, 1928.

3rd survey—Dadu, Kakar. 23rd to 29th July, 1929.

Post-epidemic period.

4th survey—Sehwan, Johi. 8th to 17th March, 1932.

5th survey—Mehar, Kakar, Johi. 10th to 19th October, 1932.

The first survey, in January 1928, was carried out by Young and Majid (1930), and the remainder by the junior author of the present paper.

GENERAL CHARACTERS OF THE DISTRICT.

Dadu District was constituted in October 1931, and is composed of seven talukas. Five of these, Mehar, Kakar, Dadu, Johi and Sehwan, formerly belonged to Larkana District, and two, Kotri and Kohistan Mahal, to Karachi District. The northern portion of Kotri taluka was formerly called Manjhand Mahal, only the southern part being known as Kotri. The localities visited in the surveys were confined to the five northern talukas. No observations were made in Kotri and Kohistan Mahal, which lie entirely outside the area commanded by the Lloyd Barrage Canals.

Dadu District is situated on the right bank of the Indus, between 25° and 27° 45' North latitude and 67° 28' and 68° 37' East longitude, and covers an area of 6,207 square miles. It is bounded on the north by Larkana District, on the south by Karachi District, and on the west by the territory of the Khan of Kalat, whilst on the east it is separated from Nawabshah and Hyderabad Districts by the River Indus.

The northern portion of the district is divided into two parts, which are entirely dissimilar in character, *viz.*, the Kohistan or hill country, and the low lands which lie between the Kohistan and the Indus. The Kohistan consists of a range of limestone hills, generally known as the Kirthar Range, which extends along the western boundary of the district, with a breadth of from twelve to fifteen miles. In Sehwan taluka they spread out and approach the Lakhi Hills, which rising near Sehwan run southwards, so that nearly the whole of the taluka is hilly. Kohistan Mahal consists of a succession of broad valleys lying between ranges of hills running generally north to south, whilst a great part of Kotri taluka consists of rocky hills and high land unfit for cultivation, though there is a strip of alluvial soil lying between the hilly portion and the Indus. The Kirthar Range consists of an ascending series of ridges running generally north and south, with broad valleys in between. The highest ridge of the range forms the boundary between Sind and Baluchistan, its general height at the northern extremity being about 5,000 feet; towards the south the height declines. There are numerous rocky torrents, the beds of which at the lower levels are dammed for purposes of irrigation.

Between the hills and the Indus, the country may be described as a broad shallow, since the middle line from north to south is considerably below the level of the Indus on one side and the base of the hills on the other. By this depression the hill torrents and the waters of the Western Nara all found their way to the Manchhar Lake and thence by the Aral River to the Indus. The soil is the rich alluvial loam of the Indus valley.

The Western Nara, which is now a canal, was at one time a loop of the Indus, the waters of which, leaving the main bed above Larkana, took a course almost parallel to it, which led them into the great natural depression which forms the Manchhar Lake. Another body of water poured into this depression

through a channel leaving the Indus south of Sehwan, called the Aral River; but when the inundation began to subside, this river flowed the other way, discharging the Manchhar into the Indus, and exposing about 20,000 acres of the richest land for cultivation in the cold season,

Under the Lloyd Barrage Scheme a flood protection embankment has been constructed, running from north to south at the foot of the Kirthar Range to divert the flood waters of the hill torrents and guide them into the Manchhar Lake. The embankment turns eastwards and passes immediately north of the lake till it reaches Sehwan, where it joins the Indus flood protection embankment. The whole of the drainage from the area commanded by the Barrage Scheme on the right bank of the Indus will also be directed into the Manchhar Lake. The bed of the Aral River, which joins the lake with the Indus, is now used to raise or lower the level of the water in the lake as required. The lower portion of the Aral, which runs immediately north of Sehwan, has been provided with a regulator, and is now used to fill the lake at the beginning of the inundation season. A new channel has been cut from the Aral to the Indus through a ridge which lies to the south of Sehwan, the cutting being 50 feet deep at one point. This channel, which is also provided with a regulator, serves to lower the level of the lake at the end of the inundation season, thus exposing a large area of rich land for cultivation.

The lake, which lies on the border between Sehwan and Johi talukas, has the shape of a long oval pointing south-west and north-east. The deep and permanent side of it is that abutting on the hills to the south-west, and its annual expansion is to the north and north-east. Before the Barrage Scheme came into operation, the lake in the inundation season covered a variable area, sometimes as much as 200 square miles. Now, the area flooded is under complete control by means of the embankment and the channels above described, and the area covered with water each year is about 60 square miles.

Climate.

Dadu District has the same seasons as the rest of Sind, but, as might be expected from its situation in the centre of the province, it is not subject to such extremes of temperature as are experienced in Upper Sind. On the other hand the climate differs very considerably from that of Karachi, in that the westerly winds of the hot season come from Kohistan and not from the sea, and consequently the heat is a dry heat.

Rainfall.

The rainfall figures recorded at the headquarter towns of the five talukas under review for the months of July to September during the period 1901-1932 are given in Table I. It will be noted that the amount of rainfall is greatest in the more southerly talukas, and that it varies very considerably in different years.

Population.

The total population of the district according to the census of 1931 was 337,153, of whom about 80 per cent are Mussulmans. The hilly part of the district is very sparsely populated, the number of persons to the square mile in Sehwan taluka being 27, whilst in Kohistan Mahal it is only five.

Cultivation.

The chief summer crops are rice, juari, and bajri, and the chief winter crop is wheat. Other crops are sesame, rape, jambo, gram, chickling vetch and bhang. The last mentioned is grown only at Bubak, its cultivation in any other part of Sind being prohibited.

Under the Lloyd Barrage Scheme the rice-growing area, which lies chiefly in the north of the district, will be supplied by the Central Rice Canal. Perennial irrigation for other parts of the district is provided by the Dadu Canal, and its branch the Johi Canal.

EPIDEMIC MALARIA.

The epidemic figures from 1901 to 1932 of the five talukas with which this paper is concerned are given in Table II. During this period there were three regional epidemics with their centre in Northern or Upper Sind, *i.e.*, 1906, 1917 and 1929, and one with its centre in Lower Sind, *i.e.*, 1906. It will be seen from the figures given that the areas under review were but slightly affected by any of these epidemics, in comparison with the figures recorded in Northern Sind. Dadu taluka seems to have suffered the worst in most instances. The causation of epidemic malaria in Sind has been discussed in detail in our previous papers (Covell and Baily, 1930*a*; 1930*b*; 1931*a*; 1931*b*; 1932), and will not be considered further here.

RESULTS OF THE SURVEYS.

1. MEHAR TALUKA.

This taluka covers an area of 327 square miles, with a population of 70,487 (in 1931), and consists of a long, narrow strip of land stretching from the Indus on the east to the Kohistan Hills on the west. The part between the river and the Western Nara is chiefly high-lying, and supports dry crops. To the west of the Nara there is a general fall in the land, and the principal crop is rice. Further west again the land once more rises to an elevated plain at the foot of the hills. The villages visited in this taluka were all situated to the west of the Nara, in the midst of the rice tract. Before the Barrage Scheme came into operation (in June 1932), the taluka was irrigated by the Western Nara and Pritchard Canals and their branches, about 94 per cent of the cultivation being under flow. The results of spleen examinations in this taluka are given in Table III.

MEHAR, the headquarters of the taluka, has a population of about 3,000, and is situated 4 miles west of the Western Nara. The cultivation immediately surrounding the village is dry crop. A minor canal distributary runs close to the village, and supplies two ponds in its midst. The subsoil water level in October 1932 was 3 to 5 feet. The spleen rate was 52 per cent (192 observations), and the average enlarged spleen was 7.3 cm.* The parasite rate was 26 per cent (100 observations, M. T., 22, B. T., 5).

* The measurements of the average enlarged spleen throughout this paper are given in terms of the distance in centimetres from the apex of the spleen to the umbilicus.

KOLACHI is a village with a population of about 450, situated 4 miles south-west of Mehar, on the bank of the Western Nara, the bed of which has here been utilized for the tail of the Central Rice Canal. Rice is cultivated within 200 yards of the village. A large pond on the eastern side of the village is fed by a canal distributary. The subsoil water level in October 1932 was 8 feet. The spleen rate was 63 per cent (82 observations), and the average enlarged spleen was 5.6 cm.

THEBA is a village with a population of about 700, situated 2 miles east of Mehar. The village is surrounded by excavations and borrow-pits, and a canal distributary flows close to it. The subsoil water level in October 1932 was 3 feet, and the chief crop grown was rice. The spleen rate was 63 per cent (100 observations), and the average enlarged spleen was 7.3 cm.

MANGWANI is a village with a population of about 750, situated 6 miles south-west of Mehar, in the midst of rice cultivation. A canal distributary flows close by the village. In October 1932 the subsoil water level was 3 feet. The spleen rate was 65 per cent (113 observations), and the average enlarged spleen was 7.5 cm. The parasite rate was 40 per cent (53 observations, M. T., 21, B. T., 2).

BANDHI, DAHOTA and BUNDHO are 3 small villages with a combined population of about 350, situated 3 miles south-west of Mehar on the bank of a minor canal, in the midst of rice cultivation. The subsoil water level in October 1932 was 6 feet. The spleen rate was 53 per cent (38 observations) and the average enlarged spleen was 6.5 cm.

SINDHAL MAHAISAR is a village with a population of about 250, situated 1 mile east of Mehar on the bank of a minor canal, and surrounded by rice cultivation. The subsoil water level in October 1932 was 5 feet. The spleen rate was 61 per cent (26 observations), and the average enlarged spleen was 7.0 cm.

BUTA SARAH is a village with a population of about 700, situated 2 miles east of Mehar and 100 yards from the Central Rice Canal, in the midst of rice cultivation. The subsoil water level in October 1932 was 8 feet. The spleen rate was 62 per cent (106 observations), and the average enlarged spleen was 6.9 cm.

THARREE MOHBAT is a village with a population of about 1,400, situated 4 miles east of Mehar, on the bank of the Western Nara Canal, in the midst of rice cultivation. The subsoil water level in October 1932 was 7 feet. The spleen rate was 47 per cent (120 observations), and the average enlarged spleen was 8.0 cm.

2. KAKAR TALUKA.

This taluka has an area of 448 square miles, with a population of 48,448 (in 1931). The western half of it consists of hill country, whilst the cultivable portion is similar to that of Mehar taluka, being high-lying in the east and west, with a depression running through the centre from north to south. Before the Lloyd Barrage Scheme came into operation the taluka was chiefly irrigated by the Western Nara, most of the cultivation being under flow. The villages visited are all situated in the rice tract which occupies the central portion of

the cultivable area. The results of spleen examinations made in the taluka are given in Table IV.

KHAIRPUR NATHAN SHAH, the headquarters of the taluka, is situated 8 miles south-west of Mchar, and about 13 miles west of the Indus. It has a population of about 1,900, and is surrounded by rice cultivation. There are two large ponds, fed by distributaries from a minor canal which flows past the village. The subsoil water level in October 1932 was 18 feet.

First survey (inter-epidemic).—In January 1928 the spleen rate was 78 (155 observations), and the average enlarged spleen was 7.5 cm.

Second survey (inter-epidemic).—In July 1929 the spleen rate was 44 per cent (220 observations), and the average enlarged spleen was 6.0 cm. The parasite rate was 22 per cent (92 observations, M. T., 7, B. T., 13, Q., 1).

Third survey (post-epidemic).—In October 1932 the spleen rate was 69 per cent (126 observations), and the average enlarged spleen was 7.4 cm. The parasite rate was 26 per cent (78 observations, M. T., 18, B. T., 2).

KAKAR is a village with a population of about 550, situated 9 miles south-west of Khairpur Nathan Shah, in the midst of rice cultivation. The village pond is fed from the Western Nara, which flows within 50 yards of it. The subsoil water level in three wells in October 1932 was between 19 and 30 feet. The spleen rate was 75 per cent (63 observations), and the average enlarged spleen was 8.7 cm. The parasite rate was 39 per cent (49 observations, M. T., 19).

WAHOURI is a village with a population of about 300, situated 8 miles south of Khairpur Nathan Shah, in the midst of rice cultivation. In October 1932 the spleen rate was 55 per cent (38 observations), and the average enlarged spleen was 6.7 cm. The parasite rate was 20 per cent (20 observations, M. T., 3, B. T., 1).

KATHIA is a village with a population of about 200, situated 7 miles south-west of Khairpur Nathan Shah, in the midst of rice cultivation, close to the Western Nara Canal. The subsoil water level was not measured, as there were no wells. In October 1932 the spleen rate was 43 per cent (75 observations), and the average enlarged spleen was 8.7 cm. The parasite rate was 26 per cent (39 observations, M. T., 6, B. T., 4).

PARYA KHOSO, DOST MOHAMED, BUK and Gozo are four small villages situated on the bank of the Western Nara Canal 4 to 6 miles west of Khairpur Nathan Shah, in the midst of rice cultivation. The tract between the canal and the hills was flooded during August 1932, and at the time of the survey in October 1932 the flood waters extended to the periphery of these villages. The subsoil water level was not ascertained, as there were no wells. The combined population of the four villages is about 650. In October 1932 the spleen rate was 54 per cent (162 observations) and the average enlarged spleen was 7.6 cm.

THALO and KANIARA are two small villages with a combined population of about 300, situated 5 miles east of Khairpur Nathan Shah on the bank of a minor canal, and in the midst of rice cultivation. In October 1932 the subsoil water level was 5 to 6 feet. The spleen rate was 70 per cent (114 observations), and the average enlarged spleen was 6.8 cm. The parasite rate was 33 per cent (45 observations, M. T., 15).

THELOO CHANDIA and HADIA KHOSO are two small villages with a combined population of about 250, situated 3 miles north-west of Khairpur Nathan Shah, in the midst of rice cultivation. The subsoil water level in October 1932 was 3 feet. The spleen rate was 57 per cent (40 observations), and the average enlarged spleen was 8.3 cm.

3. DADU TALUKA.

This taluka has an area of 297 square miles, with a population of 57,549 (in 1931), and consists of a narrow tract of alluvial land, lying between the Indus on the east and the Western Nara on the west. Before the operation of the Lloyd Barrage Scheme, the taluka was irrigated by the Western Nara and Phitowah Canals, about one-third of the cultivation being under flow. The results of spleen examinations made in the taluka are given in Table V.

DADU, the headquarters of the taluka, has a population of about 5,000, and is situated about 6 miles west of the Indus. A minor canal, which used to flow through the centre of the town, has been closed since the Barrage Scheme came into operation, and is being filled in by town rubbish. A large excavation at the north-west corner of the town, into which water was led by a canal distributary, yielded larvæ of *A. culicifacies* at the time of the survey in July 1929. The subsoil water level at that time was 19 to 20 feet, and in January 1933 it was 18 feet. There is dry crop cultivation to the east and south of the town.

In July 1929 the spleen rate was 22 per cent (130 observations), and the average enlarged spleen was 7.9 cm. The parasite rate was 27 per cent (100 observations, M. T., 20, B. T., 9).

JATOHI is a village with a population of about 250, situated 3 miles north of Dadu. There is rice cultivation within one mile of the village. The subsoil water level in July 1929 was 15 feet. The spleen rate was 22 per cent (40 observations), and the average enlarged spleen was 9.4 cm. The parasite rate was 26 per cent (35 observations, M. T., 9).

MAKHDOOM BILAWAL is a village with a population of about 500, situated about 5 miles north of Dadu, in the midst of rice cultivation. The subsoil water level in July 1929 was 6 feet. The spleen rate was 70 per cent (96 observations) and the average enlarged spleen was 7.9 cm. The parasite rate was 58 per cent (65 observations, M. T., 30, B. T., 9).

4. JOHI TALUKA.

This taluka has an area of 755 square miles, with a population of 51,435 (in 1931). It is divided into two distinct portions, the alluvial plain of the Indus valley, and the Kohistan, or hill tract. The latter depends on rain and the flood waters of mountain streams for its cultivation, whilst the former was irrigated by the Western Nara until the opening of the Lloyd Barrage Scheme. The results of spleen examinations made in this taluka are given in Table VI.

JOHI, the headquarters of the taluka, has a population of about 2,000, and is situated about 16 miles west of the Indus, in the midst of dry crop cultivation. The Johi branch of the Dadu (perennial) canal runs within $1\frac{1}{2}$ miles of the village. The subsoil water level in October 1932 was 42 feet. The

spleen rate was 5.2 per cent (192 observations), and the average enlarged spleen was 8.7 cm. The parasite rate was 22 per cent (100 observations, M. T., 19, B. T., 3).

PIR GAZI SHAH is a village with a population of about 250, situated 20 miles south-west of Johi, at the foot of the hills. The cultivation is chiefly dry crop, irrigated from a hill stream. Larvæ of *A. culicifacies* were found breeding in certain watercourses. In March 1932 the spleen rate was 23 per cent (31 observations), and the average enlarged spleen was 8.3 cm.

TANDO RAHIM KHAN is a village with a population of about 900, situated 18 miles south-west of Johi, at the foot of the hills. The cultivation is dry crop, mostly dependent on rains. The subsoil water level in March 1932 was 30 feet. The spleen rate was 12 per cent (90 observations), and the average enlarged spleen was 7.3 cm.

CHHINI is a village with a population of about 900, situated 12 miles south-west of Johi. In years of good inundation, the water of the Manchhar Lake extends to the periphery of the village. The cultivation is chiefly winter crops. In March 1932 the subsoil water level was 30 feet. The spleen rate was 7 per cent (81 observations), and the average enlarged spleen was 9.0 cm.

SHAH HUSSAIN is a village with a population of about 1,300, situated at the north-west corner of the Manchhar Lake. There were borrow-pits in the vicinity of the village containing stagnant water, but no breeding of anophelines was noted. The spleen rate in March 1932 was 1 per cent (94 observations).

BAZ MAR KHOSO is a village with a population of about 250, situated 6 miles west of Johi, in the midst of dry crops. The cultivation is dependent on rainfall, and on the flood waters of hill streams. The subsoil water level in October 1932 was 30 feet. The spleen rate was 4.3 per cent (47 observations).

HAZI KHAN is a village with a population of about 900, situated 8 miles north-west of Johi, in the midst of dry crop cultivation. The subsoil water level in October 1932 was 70 feet. The spleen rate was 9 per cent (91 observations), and the average enlarged spleen was 8.2 cm.

BHAWALPUR is a village with a population of about 850, situated 12 miles north-east of Johi. There was a certain amount of rice cultivation about half a mile from the village. The Western Nara flows within 100 yards of the village. The subsoil water level in October 1932 was 10 feet. The spleen rate was 31 per cent (111 observations), and the average enlarged spleen was 7.6 cm. The parasite rate was 17 per cent (40 observations, M. T., 5, B. T., 2).

WALWANI JAMALI is a village with a population of about 250, situated 10 miles north-east of Johi, on the banks of a drainage channel called the Lohri Dhoru. The cultivation is dry crop. In October 1932 the spleen rate was 41 (32 observations), and the average enlarged spleen was 9.1 cm.

5. SEHWAN TALUKA.

This taluka has an area of 1,273 square miles, with a population of 48,088 (in 1931). The southern half of the taluka consists of hilly country, whilst the northern portion is composed of alluvial land. A large part of this is

irrigated by the Manchhar Lake, the recession of which in the cold season exposes about 20,000 acres of land for cultivation. The remainder is irrigated by canals. The results of observations made in this taluka are given in Table VII.

SEHWAN, the headquarters of the taluka, has a population of about 4,400, and is situated on an eminence on the right bank of the Aral, which flows from the Manchhar Lake into the Indus. This portion of the Aral is now used to fill the Manchhar Lake at the beginning of the inundation season, being controlled by a regulator. A channel, also provided with a regulator, has been cut from the bed of the Aral to the Indus through a ridge which lies to the south of Sehwan. This is used to lower the level of the lake at the end of the inundation period. The bed of the Indus is about 3 miles to the east of the town, and in the inundation season its flood waters extend almost to its edge, where they are limited by a flood protection embankment. The surrounding cultivation is mainly dry crop. According to the *Gazetteer of Sind* (1927), the climate of Sehwan, with the possible exception of Jacobabad, is the worst in Sind. 'Shut in as it is by the Lakhi Range and other hills, it receives very little breeze, with the result that the heat is both concentrated and of very long duration'.

First survey (inter-epidemic).—In August 1928, the spleen rate was 14 per cent (140 observations), and the average enlarged spleen was 7.1 cm. The parasite rate was 11 per cent (100 observations, M. T., 6, B. T., 5). Crescents were observed in two of the six M. T. cases.

Second survey (post-epidemic).—In February 1934, the spleen rate was 23 per cent (214 observations).

TALTI is a village with a population of about 1,800, situated 8 miles north-west of Sehwan, in the midst of dry crop cultivation. A branch of the Phita Canal runs within 50 yards of the edge of the village. The subsoil water level in March 1932 was 23 feet. The spleen rate was 55 per cent (75 observations), and the average enlarged spleen 8.3 cm.

ARIJA is a village with a population of about 2,000, situated 7 miles north-west of Sehwan, in the midst of dry crop cultivation irrigated from the Phita Canal. In March 1932 the spleen rate was 33 per cent (116 observations), and the average enlarged spleen was 8.2 cm.

BHAN is a village with a population of about 1,400, situated 12 miles north-west of Sehwan. The cultivation is mainly dry crop, but there is a certain amount of rice cultivated at a distance of about two miles from the village. The spleen rate in March 1932 was 54 per cent (127 observations), and the average enlarged spleen was 8.2 cm.

AKATAR is a village with about 200 inhabitants, situated 9 miles north-west of Sehwan, in the midst of dry crop cultivation. Irrigation is carried out from canals, but in years of high river levels the water from the Manchhar Lake extends up to the edge of the village. In March 1932 the spleen rate was 20 per cent (40 observations), and the average enlarged spleen was 9.7 cm.

JHANGAR is a village with a population of about 1,200, situated 10 miles south-west of Sehwan, at the foot of the hills, in the midst of dry crop cultivation. The cultivation is dependent on rains and the flood waters of hill

streams. In March 1932 the spleen rate was 40 per cent (87 observations), and the average enlarged spleen was 9.1 cm.

BAJAR is a village with a population of about 900, situated 7 miles south-west of Sehwan, at the foot of the hills, and surrounded by dry crop cultivation. In years of high river levels the water from the Manchhar Lake extends to the edge of the village. In March 1932 the spleen rate was 30 per cent (63 observations), and the average enlarged spleen was 9.5 cm.

BUBAK is a village with 3,000 inhabitants, situated 9 miles west of Sehwan on the north-east bank of the Manchhar Lake, in the midst of dry crop cultivation. Bhang is also grown. The Dunster Canal runs close to the periphery of the village. In March 1932 the spleen rate was 45 per cent (223 observations), and the average enlarged spleen was 7.5 cm.

KOT BAROCHO is a village with a population of about 100, situated 10 miles south-west of Sehwan, and about $1\frac{1}{2}$ miles from the edge of the Manchhar Lake, in the midst of dry crop cultivation, which is dependent on rainfall and the flood waters of hill streams. In March 1932 the spleen rate was 16 per cent (32 observations).

TEHNI is a village with a population of about 200, situated 11 miles south-west of Sehwan, in the midst of dry crops. In March 1932 the spleen rate was 11 per cent (27 observations).

NAING is a village with about 100 inhabitants, situated 22 miles south-west of Sehwan, at the foot of the hills, in the midst of dry crop cultivation, which is dependent on rainfall and the flood waters of hill streams. In March 1932 the spleen rate was 25 per cent (16 observations).

MIANI GARKARO and MIANI ARISARAI are two temporary villages with about 400 and 600 inhabitants respectively, situated on the north bank of the Manchhar Lake, 11 miles west of Sehwan. The inhabitants, who are fishermen, move their houses in the inundation season, or else live altogether in their boats. In March 1932 the spleen rate at Miani Garkaro was 11 per cent (124 observations, average enlarged spleen 9.0 cm.), and at Miani Arisarai it was 7 per cent (95 observations, average enlarged spleen 8.6 cm.).

DISCUSSION OF RESULTS.

ENDEMIC MALARIA.

The results of our observations in Dadu District, as in other parts of Sind, have shown that the amount of endemic malaria varies widely in different areas. The localities under review in the present paper may conveniently be considered under the following headings:—

- (1) Villages in the rice tract.
- (2) Villages in the dry crop area irrigated by canals.
- (3) Villages at the foot of the hills, irrigated from hill streams and springs.
- (4) Villages in the neighbourhood of the Manchhar Lake.

Villages in the rice tract.

This is a heavily irrigated, low-lying tract, with a high subsoil water level, in places only about 3 feet below ground level in the inundation season. The

spleen results recorded in villages situated in this tract are given in Table VIII. Young and Majid (1930) visited Khairpur Nathan Shah in January 1928, i.e., just after the end of the malaria season of 1927, and found a spleen rate of 78 per cent. A number of observations were also made in certain villages in July 1929, just before the commencement of the regional epidemic of that year, i.e., at a time when one would expect the spleen rate to be at its lowest. Nevertheless, the rates were high in every case, the lowest being 43 per cent, and the highest 75.

The remaining observations were made in October 1932, i.e., 2½ years after the end of the epidemic of 1929. Again, the spleen rates were everywhere high, varying between 47 and 70 per cent. A feature of the observations recorded in the rice tract is the large size of the average enlarged spleen generally recorded. We may state, from a study of the data recorded both before and after the epidemic of 1929, that malaria is hyperendemic in this tract, the conditions resembling those found in other rice-growing areas in Sind.

Villages in the dry crop area irrigated by canals.

This tract lies between the Indus flood protection embankment and the Western Nara Canal. The spleen rates recorded are given in Table IX. Three villages were visited in this area prior to the epidemic of 1929, viz., Sehwan in August 1928, and Dadu and Jatohi in July 1929. The spleen rates were 14 per cent in Sehwan and 22 per cent in each of the other two villages.

Five villages were visited in 1932, three in March and two in October. The spleen rates varied between 31 and 55 per cent. The spleen rate recorded in Sehwan in February 1934 was 23 per cent.

As compared with the villages in the rice tract, the average enlarged spleen was considerably smaller, and the spleen rates lower. It is concluded that the amount of endemic malaria is moderate as compared with that in the rice tract, though it is considerably greater than that in the dry crop areas of Northern Sind.

Villages at the foot of the hills, irrigated from streams and springs.

The results of spleen examinations recorded in these villages are given in Table X. No observations were made in this area prior to the 1929 epidemic, all the figures given having been recorded in March or October 1932. In the latter month, a large part of this tract was submerged by floods resulting from heavy rain in the hills. Out of 7 villages visited, the spleen rate in four was less than 10 per cent, whilst in two cases only did it exceed 20 per cent. The highest figure was 25 per cent, in a village where only 16 children were examined.

It is evident that the incidence of malaria in this tract is very slight, in striking contrast with the conditions found in foothill villages in most other parts of India. This low incidence of malaria is associated with the absence of species of mosquitoes like *A. fluviatilis* (*listonii*), *A. maculatus* and *A. minimus*, which probably play the chief part in the transmission of malaria in other submontane areas. As in most other parts of Sind, *A. culicifacies* was the only malaria-carrying anopheline encountered in this area.

Villages in the neighbourhood of the Manchhar Lake.

The results of spleen examinations recorded in this area are given in Table XI. The spleen rates show considerable variations, six of them being 20 per cent or under, whilst the remaining three (Bubak, Jhangar and Bajar) yielded rates of 45, 40, and 30 per cent respectively. The lowest rate, one per cent, was recorded at Shah Hussain, which is situated at the western corner of the lake, where the margin is permanent, and the water of considerable depth at all times of the year. The rates in the two temporary fishing villages of Miani Garkaro and Miani Arisarai are also very low. It is probable that the higher rates obtaining in Bubak, Jhangar and Bajar are due to the creation of favourable breeding places for anophelines when the inundation waters recede. In the case of Bubak, the bed of the Dunster Canal may also play a part in providing breeding places. This canal is now completely cut off from the Manchhar Lake by the new flood restriction embankment.

THE REGIONAL EPIDEMIC OF 1929.

As has been already noted, Dadu District, lying as it does in Central Sind, is not as a rule seriously affected by the regional or fulminant epidemics of malaria which have their centre in Upper or Lower Sind, although it feels the effects of both to some extent. In this respect it resembles Nawabshah District, which lies in the same latitude on the opposite bank of the Indus (Covell and Baily, 1931a). Thus, in 1929, when a regional epidemic of great severity occurred in Upper Sind, the epidemic figures in the five talukas under review in no case exceeded 4.1, as compared with figures ranging from 6.2 to 15.2 in the Upper Sind Frontier District. No observations were made in Dadu District during the actual epidemic period, but the data recorded in 1932 suggest that the effects of the epidemic were chiefly felt in the alluvial tract.

**PROBABLE EFFECT OF THE LLOYD BARRAGE SCHEME ON
MALARIA IN DADU DISTRICT.**

Under the Barrage Scheme the rice tract will be irrigated by the Central Rice Canal, and other parts of the district will be irrigated by branches of the Dadu Perennial Canal. The rice area, which averaged 360,000 acres before the Barrage Scheme was inaugurated, will be increased eventually up to 560,000 acres, and this area will be surrounded on the north, north-west, south and east by perennial cultivation from the new canal system. Since the periodical lowering of the subsoil water level in the rice area was presumably due, in part at least, to the movement of water through the subsoil from areas in which the level was high to areas in which it was low, it appears possible that the new perennial irrigation which surrounds the rice area, may affect adversely the drainage capacity of the soil, and therefore cause an upward trend in the level of subsoil water, resulting eventually in water-logging (Hawes, 1932). A rise in the subsoil water level as the result of seepage from the new canals may also cause water-logging in certain areas.

Any considerable rise in the subsoil water level in this area is likely to lead to an increase in the incidence of malaria. The extent of this increase will depend on the degree of success attained by the irrigation engineers in

providing adequate drainage for the whole area on the right bank of the Indus which comes under the command of the Barrage Canals.

In some places the new canals run along the course of the original canals, which were frequently extremely tortuous. For instance, the distance from the head of the Western Nara to the Manchhar Lake is 83 miles in a straight line, but 153 miles measured along its course. In certain areas loops of the old canals will be cut off, and may act as favourable breeding areas for anopheline mosquitoes. It has been our experience everywhere in Sind that villages situated on the banks of old river beds are highly malarious, and it seems probable that any such abandoned portions of the old canals will create conditions equally favourable to a high incidence of the disease.

SUMMARY.

1. An account is given of malaria surveys carried out in various localities in Dadu District during the period 1928-1932.

2. The amount of endemic malaria varies widely in different localities. The rice tract is a hyperendemic area, and there is a considerable amount of malaria in the dry crop area between the Western Nara Canal and the Indus. The incidence of malaria is generally low in villages at the foot of the Kirthar Range of hills, and in villages situated on the margins of the Manchhar Lake.

3. The district, which lies in Central Sind, reacts only to a moderate extent to the regional epidemics which are centred in Upper and Lower Sind.

4. The possible effects of the operation of the Lloyd Barrage Scheme on the incidence of malaria in Dadu District are discussed.

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APPEN

TABLE

Rainfall figures recorded at the headquarters of 5 talukas of Dadu

Years.	MEHAR.				KAKAR.				DADU.			
	July.	August.	September.	Total.	July.	August.	September.	Total.	July.	August.	September.	Total.
1901	0.66	0.66	0.35	0.35	0.34	0.29	..	0.63
1902	..	0.95	0.54	1.49	..	1.15	0.44	1.59	..	2.38	0.70	3.08
1903	1.18	1.18	1.21	1.21	1.53	1.53
1904
1905	0.30	0.30	0.10	0.10
1906	..	0.33	..	0.33	..	0.32	..	0.32	..	1.91	..	1.91
1907	0.07	2.93	..	3.00	..	3.40	..	3.40	0.25	3.57	..	3.82
1908	0.91	1.20	..	2.11	0.79	1.16	..	1.95	1.60	0.11	..	1.71
1909	1.17	..	0.20	1.37	1.17	..	0.17	1.34	1.46	1.46
1910	2.82	0.20	..	3.02	3.97	0.07	..	4.04	4.93	0.68	..	5.61
1911	..	0.03	..	0.03	..	0.02	..	0.02
1912	0.14	0.80	..	0.94	0.14	0.20	..	0.34	0.86	0.45	..	1.31
1913	2.14	0.88	..	3.02	2.89	1.42	..	4.31	6.40	1.37	..	7.77
1914	3.88	3.88	3.26	3.26	7.01	7.01
1915	0.12	0.12	0.22	0.22	0.10	0.10
1916	..	5.10	..	5.10	..	5.79	..	5.79	..	9.35	..	9.35
1917	..	5.19	7.48	12.67	..	5.48	10.74	16.22	..	5.27	14.82	20.09
1918
1919	0.53	0.53	0.21	0.21	0.30	0.01	..	0.31
1920	0.49	0.49	0.75	0.75	0.50	0.50
1921	1.25	1.52	..	2.77	0.50	1.71	0.05	2.26	1.99	3.28	0.13	5.40
1922	..	0.05	0.68	0.73	0.21	..	0.21
1923	..	0.06	..	0.06	0.08	0.02	..	0.10	0.04	0.13	..	0.17
1924	0.24	..	2.95	3.19	0.94	..	3.65	4.59	2.75	..	4.76	7.51
1925	2.75	0.79	..	3.54	1.86	1.25	..	3.11	2.1	0.33	..	2.43
1926	..	1.32	3.96	5.28	0.88	3.35	..	4.23	..	0.41	2.80	3.21
1927	0.80	0.80	1.50	1.50	0.82	0.82
1928	0.60	0.60	0.20	0.35	..	0.55
1929	3.94	6.91	..	10.85	5.50	4.19	..	9.69	5.46	4.37	..	9.83
1930	1.71	1.71	1.53	1.53	2.80	2.80
1931	0.35	2.23	..	2.58	0.25	0.85	..	1.10	0.35	0.33	..	0.68
1932	1.95	4.30	..	5.95	4.42	1.50	..	5.92	2.49	2.91	..	5.40

DIX.

I.

District for the months July–September, 1901–1932.

JOHL.				SEHWAN.				REMARKS.
July.	August.	September	Total.	July.	August.	September.	Total.	
0.48	0.06	..	0.54	0.78	0.78	Epidemic, Upper Sind.
..	2.79	1.27	4.06	..	1.38	4.07	5.45	
1.35	1.35	2.08	2.08	
..	
0.21	0.21	
..	0.66	0.07	0.73	..	1.03	..	1.03	
1.06	2.04	..	3.10	0.41	1.74	..	2.15	
2.20	0.81	..	3.01	4.90	0.75	..	5.65	
0.87	..	1.25	2.12	4.43	..	0.47	4.90	
6.58	0.12	..	6.70	7.33	0.07	..	7.40	
..	..	0.64	0.64	..	0.13	..	0.13	
0.94	0.48	..	1.42	1.35	0.91	..	2.26	
4.81	4.05	..	8.86	7.02	1.28	0.16	8.46	
13.42	13.42	5.16	..	0.40	5.56	
0.02	..	0.11	0.13	0.25	..	0.50	0.75	Epidemic, Lower Sind.
0.09	8.51	..	8.60	3.50	8.99	..	12.49	
..	8.28	10.90	19.18	..	9.51	8.09	17.60	Epidemic, Upper Sind.
..	Epidemic, Upper Sind.
0.25	0.25	0.60	0.60	
0.60	0.60	
7.37	0.70	1.40	9.47	3.73	0.55	3.52	7.80	
..	0.02	0.47	0.49	0.32	0.16	0.01	0.49	
..	0.48	..	0.48	0.47	0.95	..	1.42	
0.44	..	3.86	4.30	1.66	..	4.25	5.91	
5.60	0.14	..	5.74	1.71	0.39	..	2.10	
..	0.95	4.15	5.10	..	2.40	..	2.40	
2.52	2.52	3.85	0.20	..	4.05	
..	1.10	..	1.10	0.90	0.90	
4.44	3.48	..	7.92	9.60	6.47	..	16.07	
2.85	2.85	3.84	3.84	
..	0.58	..	0.58	0.72	0.96	..	1.68	
1.70	1.36	..	3.06	1.86	4.55	..	6.41	

TABLE II.
Epidemic figures for 5 talukas of Dadu District, 1901-1932.

Years	Dadu.	Sehwan.	Johi.	Mehar.	Kakar.	REMARKS.
1901	3'12	2'84	3'04	2'63	2'57	Epidemic, Upper Sind.
1902	2'02	2'64	2'24	1'43	1'40	
1903	2'26	2'38	1'74	2'86	1'04	
1904	1'77	2'06	1'50	1'15	1'18	
1905 *	2'38	2'82	3'85	3'36	2'71	
1906	8'75	7'05	4'05	2'95	3'60	
1907	3'44	2'16	2'11	1'84	1'95	
1908	2'36	2'07	1'27	1'67	1'40	
1909	2'26	1'96	1'34	1'41	0'84	
1910	2'38	1'84	1'88	1'64	1'79	
1911	1'76	1'71	1'25	1'93	1'25	
1912	2'42	1'63	1'45	2'03	1'52	
1913	3'06	2'73	2'38	2'15	1'46	
1914	2'14	1'71	1'95	2'29	2'25	
1915	1'52	1'71	1'43	1'23	1'35	
1916	4'04	2'59	2'34	2'78	2'07	Epidemic, Lower Sind.
1917	6'30	6'02	6'21	7'00	4'45	Epidemic, Upper Sind.
1918	Influenza.
1919	
1920	1'11	0'48	0'97	0'91	0'84	
1921	1'53	0'96	1'59	0'92	0'07	
1922	0'98	1'70	1'23	1'13	1'46	
1923	1'03	1'00	0'88	0'66	0'50	
1924	2'44	0'96	1'75	1'18	0'70	
1925	0'98	0'77	1'32	0'86	2'13	
1926	1'39	1'21	1'89	1'30	1'29	
1927	0'92	1'48	1'41	1'17	0'82	
1928	1'27	0'94	1'56	1'04	1'84	
1929	4'08	3'04	4'11	3'11	3'09	
1930	1'33	0'67	1'27	1'02	1'14	Epidemic, Upper Sind.
1931	1'30	0'70	1'04	2'10	0'70	
1932	1'00	1'09	1'30	1'90	0'70	

TABLE III.
Results of spleen examinations carried out in Mehar taluka, 1932.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
POST-EPIDEMIC PERIOD.					
Mehar	x. 32	192	99	52'5	73
Kolachi	x. 32	82	52	63'4	69
Mangwani	x. 32	113	74	65'5	75
Theba	x. 32	100	63	63'0	73
Bandhi, Dahota and Bundho.	x. 32	38	20	52'6	65
Sindhal Mahaisar ..	x. 32	26	16	61'5	70
Buta Sarahi	x. 32	106	66	62'3	69
Tharee Mohbat ..	x. 32	120	56	46'7	80

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE IV.

Results of spleen examinations carried out in Kakar taluka, 1928-1932.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
INTER-EPIDEMIC PERIOD.					
Khairpur Nathan Shah	i. 28	155	113	78.1	7.5
Khairpur Nathan Shah	vii. 29	220	98	44.5	6.0
Kakar	vii. 29	63	47	74.6	8.7
Wahouri	vii. 29	38	21	55.3	6.7
Kathia	vii. 29	75	32	42.7	8.7
POST-EPIDEMIC PERIOD.					
Khairpur Nathan Shah	x. 32	126	87	69.0	7.4
Parva Khoso, Dost Mohd, Buk and Gozo	x. 32	162	88	54.3	7.6
Thalo and Kaniara ..	x. 32	114	80	70.1	6.8
Theloo Chandia and Hadia Khoso.	x. 32	40	23	57.5	8.3

TABLE V.

Results of spleen examinations carried out in Dadu taluka, 1929.

INTER-EPIDEMIC PERIOD					
Dadu ..	vii. 29	130	29	22.3	7.9
Jatohi ..	vii. 29	40	9	22.5	9.4
Makhdoom Bilawal	vii. 29	96	67	69.8	7.9

TABLE VI.

Results of spleen examinations carried out in Johi taluka, 1932.

POST-EPIDEMIC PERIOD.					
Pir G. Shah ..	iii. 32	31	7	22.6	8.3
Tando Maim Khan	iii. 32	90	11	12.2	7.3
Chhini ..	iii. 32	81	6	7.4	9.0
Shah Hussain ..	iii. 32	94	1	1.0	..
Baz Mar Khoso ..	x. 32	47	2	4.3	..
Hazi Khan ..	x. 32	91	8	8.8	8.2
Bhawalpur ..	x. 32	111	35	31.5	7.6
Walwani Jamali ..	x. 32	32	13	40.6	9.1
Johi ..	x. 32	192	10	5.2	8.7

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE VII.
Results of spleen examinations in Sehwan taluka, 1928-1932.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
INTER-EPIDEMIC PERIOD.					
Sehwan	viii. 28	140	20	14.3	7.1
POST-EPIDEMIC PERIOD.					
Talti	iii. 32	75	41	54.6	8.3
Arija	iii. 32	116	32	32.7	8.2
Bhan	iii. 32	127	69	54.3	8.2
Akatar	iii. 32	40	8	20.0	9.7
Jhangar	iii. 32	87	35	40.2	9.1
Bajar	iii. 32	63	19	30.2	9.5
Bubak	iii. 32	100	45	45.0	7.5
Kot Barocho	iii. 32	32	5	15.6	..
Tehni	iii. 32	27	3	11.1	..
Naing	iii. 32	16	4	25.0	..
Miani Garkaro	iii. 32	124	13	10.5	9.0
Miani Arisarai	iii. 32	95	7	7.4	8.6
Sehwan	ii. 34	214	49	22.9	.

TABLE VIII.
Results of spleen examinations in the rice tract of Dadu District.

INTER-EPIDEMIC PERIOD.					
Khairpur Nathan Shah	i. 28	155	113	78.1	7.5
Khairpur Nathan Shah	vii. 29	220	98	44.5	6.0
Kakar	vii. 29	63	47	74.6	8.7
Wahouri	vii. 29	38	21	55.3	6.7
Kathia	vii. 29	75	32	42.7	8.7
Makhdoom Bilawal ..	vii. 29	96	67	69.8	7.9
POST-EPIDEMIC PERIOD.					
Khairpur Nathan Shah	x. 32	126	87	69.0	7.4
Parya Khoso, Dost	x. 32	162	88	54.3	7.6
Mohd, Buk and Gozo.					
Thalo and Kaniara ..	x. 32	114	80	70.1	6.8
Theloo Chandia and	x. 32	40	23	57.5	8.3
Hadia Khoso.					
Mehar	x. 32	192	99	52.5	7.3
Kolachi	x. 32	82	52	63.4	6.9
Mangwani	x. 32	113	74	65.5	7.5
Theba	x. 32	100	63	63.0	7.3
Bandhi, Dahota and	x. 32	38	20	52.6	6.5
Bundho.					
Sindhal Mahaisar ..	x. 32	26	16	61.5	7.0
Buta Sarahi	x. 32	106	66	62.3	6.9
Tharee Mohbat	x. 32	120	56	46.7	8.0

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE IX.

Results of spleen examinations in the dry crop area of Dadu District irrigated by inundation canals before the Barrage Scheme.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
INTER-EPIDEMIC PERIOD.					
Sehwan	viii. 28	140	20	14.3	7.1
Dadu	vii. 29	130	29	22.3	7.9
Jatohi	vii. 29	40	9	22.5	9.4
POST-EPIDEMIC PERIOD.					
Arija ..	iii. 32	116	32	32.7	8.2
Talti ..	iii. 32	75	41	54.6	8.3
Bhan ..	iii. 32	127	69	54.3	8.2
Bhawalpur	x. 32	111	35	31.5	7.6
Wulwani Jamali	x. 32	32	13	40.6	9.1
Schwan ..	ii. 34	214	49	22.9	..

TABLE X.

Results of spleen examinations in areas irrigated from hill streams and springs in Dadu District, 1932.

POST-EPIDEMIC PERIOD.					
Pir Gazi Shah	iii. 32	31	7	22.6	8.3
Tando Rahim Khan	iii. 32	90	11	12.2	7.3
Chhini ..	iii. 32	81	6	7.4	9.0
Naing ..	iii. 32	16	4	25.0	..
Baz Mar Khoso	x. 32	47	2	4.3	..
Hazi Khan	x. 32	91	8	8.8	8.2
Johi ..	x. 32	192	10	5.2	8.7

TABLE XI.

Results of spleen examinations in villages situated in the neighbourhood of the Manchhar Lake, 1932.

POST-EPIDEMIC PERIOD.					
Akatar ..	iii. 32	40	8	20.0	9.7
Jhangar ..	iii. 32	87	35	40.2	9.1
Bajar ..	iii. 32	63	19	30.2	9.5
Kot Barocho	iii. 32	32	5	15.6	..
Bubak ..	iii. 32	100	45	45.0	7.5
Tehni ..	iii. 32	27	3	11.1	..
Miani Garkaro	iii. 32	124	13	10.5	9.0
Miani Arisarai	iii. 32	95	7	7.4	8.6
Shah Hussain	iii. 32	94	1	1.0	..

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE XII.

Results of blood examinations made in Dadu District.

Locality.	Date.	Number ex- amined	Number with parasites.	Parasite rate.	M. T. infection rate.	B. T. infection rate.	Q. infection rate.
INTER-EPIDEMIC PERIOD.							
Sehwan ..	viii. 28	100	11	11'0	6'0	5'0	0'0
Dadu ..	vii. 29	100	27	27'0	20'0	9'0	0'0
Jatohi ..	vii. 29	35	9	25'7	25'7	0'0	0'0
Makhdoom Bilawal	vii. 29	65	38	58'4	46'1	13'8	0'0
Kakar ..	vii. 29	49	19	38'9	38'9	0'0	0'0
Wahouri ..	vii. 29	20	4	20'0	15'0	5'0	0'0
Kathia ..	vii. 29	39	10	25'6	15'4	10'3	0'0
Khairpur Nathan Shah	vii. 29	92	20	21'7	7'6	14'1	1'1
POST-EPIDEMIC PERIOD.							
Khairpur Nathan Shah	x. 32	78	20	25'6	23'1	2'6	0'0
Thalo ..	x. 32	27	9	33'3	33'3	0'0	0'0
Kaniara ..	x. 32	18	6	33'3	33'3	0'0	0'0
Mangwani ..	x. 32	53	21	39'6	39'6	3'8	0'0
Mehar ..	x. 32	100	26	26'0	22'0	5'0	0'0
Bhawalpur ..	x. 32	40	7	17'5	12'5	5'0	0'0
Johi ..	x. 32	100	22	22'0	19'0	3'0	0'0

MALARIA IN SIND.

Part XI.

MALARIA IN LARKANA DISTRICT.

BY

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[30th January, 1934.]

INTRODUCTION.

PERIOD AND SCOPE OF SURVEYS.

THE investigations on which the present paper is based were made for the most part during two visits to the district, *viz.*, in the spring of 1927, and in the autumn of 1932. A severe regional epidemic of malaria occurred throughout Upper Sind in the autumn of 1929, so that the survey of 1927, which was carried out by Young and Majid (1930), was made in the inter-epidemic period, whilst the second survey, which was carried out by the junior author of the present paper, was made in the post-epidemic period.

The names of the talukas visited, and the periods at which the surveys were made, are given below :—

Inter-epidemic period.

1st survey—Larkana, Ratodero, Mirokhan, Kambar, Labdarya. April 1927.

2nd survey—Larkana, Ratodero, Mirokhan, Kambar, Labdarya, Warah. November to December 1932.

Observations made in two villages of Larkana taluka, and in a group of six villages in Kambar taluka, which formed the subject of special studies, have been recorded in two of our previous papers (Covell and Baily, 1930; 1931a).

GENERAL CHARACTERS OF THE DISTRICT.

Larkana District is situated between $27^{\circ} 14'$ and 28° North latitude and $67^{\circ} 11'$ and $68^{\circ} 33'$ East longitude, on the right bank of the River Indus, and covers an area of 2,576 square miles. Formerly, the district consisted of 11 talukas, but in 1931 the five most southerly talukas were withdrawn to form part of the new Dadu District, whilst one of the talukas of the Upper Sind Frontier District, Shahdadtal taluka, was added to Larkana District. This now consists of the seven talukas of Shahdadtal, Ratodero, Mirokhan, Larkana, Kambar, Warah and Labdarya. Observations carried out in Shahdadtal taluka in 1930 were included in our paper dealing with malaria in the Upper Sind Frontier District (Covell and Baily, 1931b). The present paper deals with the remaining six talukas only.

The district is bounded on the north by the Upper Sind Frontier and Sukkur Districts; on the west by the territory of the Khan of Kalat; on the south by Dadu District; whilst on the east it is separated from Kairpur State and the northern part of Nawabshah District by the River Indus. It is divided into two parts, which are entirely dissimilar in character, the Kohistan or hill country on the west, and the low lands which lie between the Kohistan and the Indus. All the localities dealt with in the present paper lie in the latter tract.

There are, properly speaking, no rivers in the district except the Indus. The Western Nara, which was formerly a loop of the Indus, taking off from the main bed above Larkana, is now a canal. There are however numerous watercourses which drain the hills, and are known as 'nais'. These are fierce torrents after rain, but they would speedily dry up when the rain ceased if they were not dammed for purposes of cultivation.

Climate.

The climate of Larkana District is said to be the most severe in Sind, next to that in the Upper Sind Frontier District. The great heat of the summer months is little mitigated by even such breezes as visit Sukkur from off the river at night, whilst the ubiquitous canals and general submergence of the country add moisture to the heat (*Gazetteer of the Province of Sind*, 1927). The maximum and minimum temperatures recorded at Larkana town during the months of January, May, July and November for a number of years are given in Table II.

Rainfall.

The mean annual rainfall is about three inches, but it varies considerably in different years. The amount of rainfall recorded at the headquarter towns of five of the six talukas under review for a number of years is given in Table I. The figures for Mirokhan taluka are not available.

Population.

The total population of the district in 1931 was 458,557. Larkana is the most populous district in Sind, the most densely populated region being Larkana taluka itself, which had 279 inhabitants to the square mile in 1921. Of the total population about 83 per cent are Mussalmans.

Cultivation.

Of the summer crops, rice is the chief, and is indeed the staple crop of the district. The major part of the great rice tract of Sind lies in Larkana District, and under the Lloyd Barrage Scheme this is irrigated by the Central Rice Canal and its branches. Juari, and to a less extent bajri, and sesame are grown in every taluka. Of the winter crops, wheat has hitherto been grown in every taluka on lands which have been submerged by the spill of a canal (*sailabi* cultivation), or on lands which have been given a flooding towards the end of the inundation (*bosi* cultivation). Rape and jhambo are also grown, and gram and chickling vetch are cultivated as second crops in rice lands throughout the district, and as winter crops in the riverain tracts.

EPIDEMIC MALARIA.

The epidemic figures recorded from 1901 to 1932 in the six talukas dealt with in the present paper are given in Table III. During this period there were three regional epidemics with their centre in Upper Sind, *viz.*, in 1906, 1917 and 1929. In 1906 Larkana District was but slightly affected, only one taluka showing a figure of over five. The epidemic of 1917 was much more severely felt, figures of over eight being recorded in five of the six talukas. In 1929 the figures were not so high, though three of the talukas had epidemic figures of more than five. On the whole, it may be said that whilst the effects of the regional epidemics of Northern Sind are felt throughout Larkana District, yet the mortality occasioned by them is considerably less than is the case in the Upper Sind Frontier and Sukkur Districts.

RESULTS OF SURVEYS.

1. RATODERO TALUKA.

This taluka has an area of 233 square miles with a population of 59,781 (in 1931). It consists of an alluvial tract of land, irrigated, before the operation of the Lloyd Barrage Scheme, by the Ghar and Sukkur Canals, over 90 per cent of the cultivation being by flow. The villages visited were situated in the rice-growing tract between Nao Dero and Ratodero, which is the most fertile part of the taluka. A strip of land lying to the east of the taluka is subject to annual flooding from the Indus in the inundation season. The results of spleen examinations made in this taluka are given in Table IV.

RATODERO, the headquarters of the taluka, has a population of about 7,300, and is situated in the midst of rice cultivation 10 miles north of Nao Dero. There is also some date palm cultivation in the vicinity. The Sukkur Canal flows close by the town, which is surrounded by numerous excavations and borrow-pits. In November 1932 the subsoil water level was 8 to 10 feet. Adult specimens of *A. culicifacies*, *A. subpictus* and *A. pulcherrimus* were

caught. The spleen rate was 57 per cent (295 observations), and the average enlarged spleen was 7.7 cm.* The parasite rate was 29 per cent (100 observations, M. T., 25, B. T., 6). Crescents were observed in 7 cases.

NAO DERO is a village with a population of about 2,000, situated 10 miles south-east of Ratodero, on the bank of the Ghar Canal, surrounded by dry crop cultivation. The subsoil water level in November 1929 was 8 to 10 feet. Larvæ of *A. culicifacies* were caught in the village pond, and adult specimens of *A. culicifacies*, *A. subpictus* and *A. pulcherrimus* were captured.

First survey (inter-epidemic).—In April 1927 the spleen rate was 22.5 per cent (146 observations).

Second survey (inter-epidemic).—In November 1927 the spleen rate was 30 per cent (100 observations), and the average enlarged spleen was 7.9 cm. The parasite rate was 14 per cent (90 observations, M. T., 7, B. T., 6). Crescents were observed in 5 cases.

Third survey (inter-epidemic).—In July 1928 the spleen rate was 13 per cent (90 observations), and the average enlarged spleen was 7.4 cm. The parasite rate was 10 per cent (90 observations, M. T., 7, B. T., 2). Crescents were observed in one case.

Fourth survey (post-epidemic).—In November 1932 the spleen rate was 65 per cent (145 observations), and the average enlarged spleen was 6.6 cm. The parasite rate was 35 per cent (20 observations, M. T., 7). Crescents were observed in 3 cases.

PIR JO GOR is a village with a population of about 1,100, situated on the bank of the Ghar Canal one mile south of Nao Dero, in the midst of date palm cultivation, and with rice cultivation within about 500 yards. The subsoil water level in November 1932 was 9 feet. Larvæ of *A. culicifacies* were found in certain irrigation wells, and adult specimens of *A. culicifacies*, *A. subpictus* and *A. pulcherrimus* were collected in the village. The spleen rate was 68 per cent (100 observations), and the average enlarged spleen was 5.9 cm. The parasite rate was 40 per cent (20 observations, M. T., 8).

PANJOO DERO is a village with about 1,300 inhabitants, situated 10 miles south-east of Ratodero, in the midst of rice cultivation. The Central Rice Canal runs within 300 yards of the village, and a canal distributary runs within 50 yards of its periphery. The subsoil water level in November 1932 was 9 feet. Adult specimens of *A. culicifacies*, *A. subpictus* and *A. pulcherrimus* were collected. The spleen rate was 82 per cent (88 observations), and the average enlarged spleen was 7.5 cm. The parasite rate was 40 per cent (10 observations, M. T., 4). Crescents were observed in one case.

KHAN WAH is a village with a population of about 550, situated 8 miles south of Ratodero, in the midst of rice cultivation, which extends to within 400 yards of its edge. In November 1932 the subsoil water level in the village was 10 feet, but in a well near the rice cultivation it was only 4 feet. Adult specimens of *A. subpictus*, *A. culicifacies*, *A. pulcherrimus* and *A. stephensi* were collected in the village. The spleen rate was 63 per cent (73 observations),

* The measurement of the average enlarged spleen throughout this paper is given in terms of the distance in centimetres from the apex of the spleen to the umbilicus.

and the average enlarged spleen was 7.2 cm. The parasite rate was 44 per cent (25 observations, M. T., 10, B. T., 1). Crescents were observed in 3 cases.

PIR BAKSH BHUTO is a village with a population of about 850, situated 4 miles south-east of Ratodero, in the midst of rice cultivation. In November 1932 the subsoil water level was 6 to 7 feet. Larvæ of *A. culicifacies* were collected from one of the village ponds. The spleen rate was 66 per cent (67 observations), and the average enlarged spleen was 7.7 cm.

MASU DERO is a village with about 600 inhabitants, situated 6 miles south-east of Ratodero, in the midst of rice cultivation. The subsoil water level in November 1932 was 6 to 10 feet. Adult specimens of *A. culicifacies*, *A. subpictus* and *A. pulcherrimus* were collected in the village. The spleen rate was 67 per cent (40 observations), and the average enlarged spleen was 8.0 cm. The parasite rate was 28 per cent (25 observations, M. T., 7). Crescents were observed in two cases.

GHARI KHUDA BAKSH is a village with a population of about 450, situated 10 miles south of Ratodero, in the midst of rice cultivation. The subsoil water level in November 1932 was 5 feet. Adult specimens of *A. subpictus*, *A. culicifacies* and *A. pulcherrimus* were collected in the village. The spleen rate was 53 per cent (55 observations), and the average enlarged spleen was 6.4 cm. The parasite rate was 52 per cent (25 observations, M. T., 12, B. T., 1). Crescents were observed in 6 cases.

BUNGAL DERO is a village with about 2,100 inhabitants, situated 10 miles south of Ratodero, in the midst of rice cultivation. The subsoil water level in November 1932 was 9 feet. The spleen rate was 59 per cent (276 observations), and the average enlarged spleen was 6.5 cm.

2. MIROKHAN TALUKA.

This taluka has an area of 288 square miles, with a population of 47,214 (in 1931). Before the operation of the Lloyd Barrage Scheme it was irrigated by the Sukkur Canal and by branches of the Ghar Canal, the cultivation being almost entirely by flow. Large tracts are affected by 'kalar' (deposits of salt) and sand, and are consequently uncultivated. The taluka is somewhat sparsely inhabited, and the villages are scattered. The results of spleen examinations made in this taluka are given in Table V.

MIROKHAN, the headquarters of the taluka, has a population of about 2,000, and is situated 16 miles north-west of Larkana, in the midst of rice cultivation. The subsoil water level in November 1932 was 10 to 16 feet. Adult specimens of *A. subpictus*, *A. culicifacies*, *A. pulcherrimus* and *A. stephensi* were collected in the village. The spleen rate was 50 per cent (80 observations), and the average enlarged spleen was 8.4 cm. The parasite rate was 18 per cent (50 observations, M. T., 9). Crescents were observed in one case.

JOYA is a village with a population of about 450, situated 6 miles south-west of Mirokhan, in the midst of rice cultivation, close to a minor canal. In November 1932 the subsoil water level was 6 to 8 feet. The spleen rate was 61 per cent (65 observations), and the average enlarged spleen was 6.1 cm.

The parasite rate was 24 per cent (25 observations, M. T., 6). Crescents were observed in 3 cases.

LANGHARI (LAL BAKSH) is a village with about 200 inhabitants, situated 5 miles south-west of Mirokhan, in the midst of rice cultivation. In November 1932 the subsoil water level was 10 feet. The spleen rate was 44 per cent (18 observations), and the average enlarged spleen was 7.9 cm.

THARO WADIYO is a village with a population of about 800, situated 3 miles south-west of Mirokhan, and surrounded by dry crop cultivation, though there is a certain amount of rice grown 500 yards from its edge. In November 1932 the subsoil water level was 6 feet. Adult specimens of *A. culicifacies*, *A. subpictus*, *A. pulcherrimus*, *A. stephensi* and *A. hyrcanus* var. *nigerrimus* were collected in the village. The spleen rate was 77 per cent (40 observations), and the average enlarged spleen was 6.7 cm.

SUJAWAL is a village with a population of about 550, situated 8 miles north of Mirokhan, in the midst of rice cultivation, which extends to within 300 yards of its margin. In November 1932 the subsoil water level was 13 feet. The spleen rate was 60 per cent (102 observations), and the average enlarged spleen was 6.8 cm. The parasite rate was 28 per cent (25 observations, M. T., 7).

CHOWSOOL is a village with a population of about 400, situated 7 miles south-east of Mirokhan, in the midst of rice cultivation. In November 1932 the subsoil water level was 7 feet.

First survey (inter-epidemic).—In April 1927 there was no rice cultivation around this village, as the area had not received sufficient water for this purpose during the previous three years. The spleen rate was 3 per cent (33 observations).

Second survey (early post-epidemic).—In March 1931 the spleen rate was 81 per cent (43 observations), and the average enlarged spleen was 7.0 cm.

Third survey (post-epidemic).—In November 1932 the spleen rate was 79 per cent (47 observations), and the average enlarged spleen was 6.0 cm. The parasite rate was 32 per cent (19 observations, M. T., 6). Crescents were observed in one case.

BANGLOW MAHBAB is a village with a population of about 450, situated 6 miles south-east of Mirokhan, in the midst of rice cultivation, which extends to within 400 yards of its edge. In November 1932 the subsoil water level was 7 feet. The spleen rate was 69 per cent (58 observations), and the average enlarged spleen was 7.0 cm.

BUTHI is a village with about 1,300 inhabitants, situated 6 miles south-west of Mirokhan, in the midst of rice cultivation, though at the time of our visit (November 1932) this was less than usual owing to scarcity of water in that year. The subsoil water level was 6 to 8 feet. The spleen rate was 72 per cent (58 observations), and the average enlarged spleen was 6.2 cm.

BAHRAM HETHIAN is a village with a population of about 450, situated 8 miles south-west of Mirokhan. There is no cultivation within 400 yards of the margin of the village, but beyond this limit rice is grown. In November

1932 the subsoil water level was 10 feet. The spleen rate was 60 per cent (30 observations), and the average enlarged spleen was 8.4 cm.

3. KAMBAR TALUKA.

This taluka has an area of 473 square miles, with a population of 81,745 (in 1931). It is one of the most fertile talukas in Sind, although to the west of Kambar town there is an extensive plain of salt-impregnated land as bad as any in the province. The western portion of the taluka is hilly, depending for its water supply on hill streams and rainfall. The remainder of the taluka contains the best rice lands in the province. The tract to the south of Kambar, including a portion of Larkana taluka, is known as the 'Mail' country, and is particularly famous, the village of Ghogharo having a special reputation for the quality of its rice. The water supply, before the operation of the Lloyd Barrage Scheme, was derived entirely from the Ghar Canal and its branches, the cultivation being entirely by flow. The results of spleen examinations made in this taluka are given in Table VI.

KAMBAR, the headquarters of the taluka, has a population of about 9,300, and is situated 9 miles west of Larkana. To the north and east of the town there is rice cultivation, and also some date palm groves. A large pond, which used to be in the middle of the town, has been filled in since 1931. In November 1932 the subsoil water level was 5 to 10 feet. Adult specimens of *A. pulcherrimus*, *A. culicifacies*, *A. subpictus*, *A. stephensi* and *A. hyrcanus* var. *nigerrimus* were collected in the town.

First survey (inter-epidemic).—In April 1927 the spleen rate was 45 per cent (126 observations).

Second survey (inter-epidemic)—In November 1927 the spleen rate was 45 per cent (112 observations), and the average enlarged spleen was 8.5 cm. The parasite rate was 35 per cent (112 observations, M. T., 16, B. T., 19). Crescents were observed in 5 cases.

Third survey (inter-epidemic).—In July 1928 the spleen rate was 37 per cent (100 observations), and the average enlarged spleen was 5.6 cm. The parasite rate was 35 per cent (100 observations, M. T., 31, B. T., 4, Q., 1).

Fourth survey (height of epidemic).—In November 1929 the spleen rate was 82 per cent (147 observations), and the average enlarged spleen was 7.2 cm. The parasite rate was 84 per cent (50 observations, M. T., 42). Crescents were observed in 2 cases.

Fifth survey (post-epidemic).—In November 1932 the spleen rate was 48 per cent (200 observations), and the average enlarged spleen was 7.6 cm. The parasite rate was 9 per cent (100 observations, M. T., 9). Crescents were observed in 4 cases.

BHER is a village with a population of about 1,100, situated 4 miles south-west of Kambar, in the midst of rice cultivation. A large lake or 'dhand', called Changro, extends almost to the edge of the village. In November 1932 the subsoil water level was 4½ feet.

First survey (inter-epidemic).—In January 1928 the spleen rate was 77 per cent (83 observations), and the average enlarged spleen was 7.1 cm.

The parasite rate was 29 per cent (83 observations, M. T., 14, Q., 10). Crescents were observed in 10 cases.

Second survey (inter-epidemic).—In July 1928 the spleen rate was 54 per cent (50 observations), and the average enlarged spleen was 7.0 cm. The parasite rate was 10 per cent (50 observations, M. T., 5). Crescents were observed in 10 cases.

Third survey (height of epidemic).—In November 1929 the spleen rate was 84 per cent (82 observations), and the average enlarged spleen was 7.4 cm. The parasite rate was 80 per cent (50 observations, M. T., 37, B. T., 3). Crescents were observed in 4 cases.

Fourth survey (post-epidemic).—In November 1932 the spleen rate was 57 per cent (110 observations), and the average enlarged spleen was 7.4 cm.

GHATAHAR is a village with about 1,800 inhabitants, situated 5 miles south-west of Kambar, in the midst of rice cultivation. The subsoil water level in November 1932 was 16 feet. The spleen rate was 59 per cent (115 observations), and the average enlarged spleen was 7.4 cm. The parasite rate was 26 per cent (43 observations, M. T., 11). Crescents were observed in one case.

HANI is a village with about 800 inhabitants, situated 6 miles south-west of Kambar, in the midst of rice cultivation. A minor canal runs close by the village. In November 1932 the subsoil water level was 9 feet. The spleen rate was 66 per cent (47 observations), and the average enlarged spleen was 7.0 cm.

MENA is a village with a population of about 1,200, situated 7 miles north-east of Kambar, in the midst of rice cultivation. In November 1932 the subsoil water level was 6 feet. The spleen rate was 58 per cent (83 observations), and the average enlarged spleen was 7.7 cm.

HULIA is a village with a population of about 250, situated 4 miles north-east of Kambar, in the midst of rice cultivation. In November 1932 the subsoil water level was 5 feet. The spleen rate was 24 per cent (45 observations), and the average enlarged spleen was 8.4 cm.

GOT NUR MOHAMED KHAN is a village with about 650 inhabitants, situated 7 miles west of Kambar. The land immediately surrounding the village is uncultivated, but there is rice cultivation within about 500 yards. In November 1932 the spleen rate was 60 per cent (40 observations), and the average enlarged spleen was 7.4 cm. The subsoil water level was 5 feet.

MEHAN JI KHAI is a village with a population of about 550, situated 6 miles west of Kambar, in the midst of rice cultivation, which extends to within 400 yards of its periphery. A minor canal, the Nur Wah, flows past the village. In November 1932 the spleen rate was 72 per cent (67 observations), and the average enlarged spleen was 7.0 cm.

DAPHAR is a village with a population of about 500, situated 5 miles west of Kambar. The chief cultivation is rice, which extends to within 500 yards of the edge of the village. In November 1932 the spleen rate was 59 (34 observations), and the average enlarged spleen was 8.1 cm.

4. WARAH TALUKA.

This taluka has an area of 418 miles, with a population of 64,783 (in 1931). Before the operation of the Lloyd Barrage Scheme it was mainly irrigated by the Ghar Canal system and some minor canals of the Western Nara system, almost all the cultivation being under flow. The eastern portion of the taluka was well cultivated and prosperous: the western, owing to its irregular supply of water, was chiefly waste. The results of spleen examinations made in this taluka are given in Table VII.

WARAH, the headquarters of the taluka, has a population of about 1,200, and is situated on the bank of a minor canal, the Nekah Wah, 16 miles south-west of Kambar, in the midst of rice cultivation. In November 1932 the subsoil water level was 4 feet. Adult specimens of *A. culicifacies*, *A. pulcherrimus* and *A. subpictus* were collected in the village. The spleen rate was 79 per cent (185 observations), and the average enlarged spleen was 7.0 cm.

NASIRABAD is a village with about 1,600 inhabitants, situated 9 miles south-east of Warah, on the bank of a minor branch of the Ghar Canal, in the midst of rice cultivation, which extends to within 350 yards of its periphery. In November 1932 the subsoil water level was 7 feet. Adult specimens of *A. culicifacies*, *A. pulcherrimus* and *A. subpictus* were collected in the village.

First survey (inter-epidemic).—In January 1928 the spleen rate was 74 per cent.

Second survey (post-epidemic).—In November 1932 the spleen rate was 60 per cent (222 observations), and the average enlarged spleen was 6.9 cm. The parasite rate was 26 per cent (50 observations, M. T., 13). Crescents were observed in one case.

PANHWAR and MURADI are two small villages with a combined population of about 450, situated 7 miles south-east of Warah, in the midst of rice cultivation. In November 1932 the spleen rate was 50 per cent (30 observations), and the average enlarged spleen was 7.5 cm.

LAKHA is a village with a population of about 800, situated 6 miles south-east of Warah, in the midst of rice cultivation. In November 1932 the subsoil water level was 10 feet. The spleen rate was 60 per cent (72 observations), and the average enlarged spleen was 7.5 cm.

GHAZI KHUHAWAR is a village with a population of about 1,600, situated 7 miles south of Warah, in the midst of rice cultivation. Juari is also grown in the neighbourhood. In November 1932 the subsoil water level was 4 feet. The spleen rate was 40 per cent (252 observations), and the average enlarged spleen was 8.0 cm. The parasite rate was 24 per cent (34 observations, M. T., 8). Crescents were observed in one case.

JANI JO BAND is a village with about 600 inhabitants, situated 7 miles east of Warah, in the midst of rice cultivation. The subsoil water level in November 1932 was 9 feet. The spleen rate was 58 per cent (108 observations), and the average enlarged spleen was 7.5 cm.

HAITAM SAHO is a village with a population of about 200, situated 5 miles east of Warah, in the midst of rice cultivation. In November 1932 the subsoil

water level was 7 feet. The spleen rate was 29 per cent (55 observations), and the average enlarged spleen was 8.1 cm.

WAGAN is a village with a population of about 1,350, situated 12 miles east of Warah, in the midst of rice cultivation. In November 1932 the subsoil water level was 11 feet. The spleen rate was 74 per cent (125 observations), and the average enlarged spleen was 8.1 cm. The parasite rate was 13 per cent (61 observations, M. T., 8).

5. LABDARYA TALUKA.

This taluka has an area of 326 square miles, with a population of 76,567 (in 1931). Before the operation of the Lloyd Barrage Scheme the taluka was irrigated from the Western Nara, which runs through it from north to south, 96 per cent of the cultivation being by flow. Although it is not quite so fertile as Larkana and Kambar talukas, it nevertheless produces very good crops. Between the flood restriction embankment and the River Indus, the land is extensively cultivated with wheat and other winter crops. The western portion of the taluka forms part of the rice tract, and it was in this area that most of the villages visited were situated. Trees grow extremely well in this taluka, and the long avenues of Nim, Siriah and Babul at Bakrani, Dokri and Badeh are the finest in the province. Mango groves and gardens are more numerous in this taluka than anywhere else in the district. The results of spleen examinations made in the taluka are given in Table VIII.

DOKRI, the headquarters of the taluka, has a population of about 1,700 and is situated 14 miles south of Larkana, on the banks of the Western Nara Canal, in the midst of rice cultivation. The subsoil water level in November 1932 was 12 feet. Adult specimens of *A. subpictus*, *A. pulcherrimus* and *A. culicifacies* were collected in the village.

First survey (inter-epidemic).—In April 1927 the spleen rate was 48 per cent (56 observations).

Second survey (post-epidemic).—In November 1932 the spleen rate was 86 per cent (151 observations), and the average enlarged spleen was 6.2 cm. The parasite rate was 32 per cent (80 observations, M. T., 25). Crescents were observed in 6 cases.

BADEH is a village with a population of about 2,000, situated 6 miles south-west of Dokri, in the midst of rice cultivation. The subsoil water level in November 1932 was 8 feet. The spleen rate was 54 per cent (300 observations), and the average enlarged spleen was 7.1 cm. The parasite rate was 26 per cent (50 observations, M. T., 13). Crescents were observed in 2 cases.

DARRA is a village with about 700 inhabitants, situated 3 miles south-west of Dokri, in the midst of rice cultivation. The subsoil water level in November 1932 was 6 feet. The spleen rate was 72 per cent (36 observations), and the average enlarged spleen was 6.8 cm.

GERELO is a village with a population of about 1,800, situated 6 miles north of Dokri, in the midst of rice cultivation. In November 1932 the subsoil water level was 8 feet. The spleen rate was 68 per cent (220 observations), and the average enlarged spleen was 6.4 cm.

THARECHA is a village with about 500 inhabitants, situated 5 miles north of Dokri, in close proximity to a minor canal, in the midst of rice cultivation. In November 1932 the subsoil water level was 8 feet. The spleen rate was 84 per cent (58 observations), and the average enlarged spleen was 7.5 cm.

MAD BAHU is a village with a population of about 1,650, situated 9 miles north-east of Dokri, surrounded by dry crop cultivation. In November 1932 the subsoil water level was 10 feet. The spleen rate was 83 per cent (105 observations), and the average enlarged spleen was 7.0 cm.

6. LARKANA TALUKA.

This taluka has an area of 219 square miles, with a population of 95,111 (in 1931). It may be divided into two parts, one between the River Indus and the flood restriction embankments, and the other on the further side of these embankments. The former tract is liable to flooding from the river in the inundation season, and winter crops, especially wheat, are for the most part grown. The latter forms part of the richest and best cultivated land in Sind. The cultivation, before the Lloyd Barrage Scheme came into operation, was almost entirely by flow from the Ghar Canal and the Western Nara, and rice is the predominant crop. The whole country is studded with prosperous villages. The results of spleen examinations made in this taluka are given in Table IX.

LARKANA, the headquarters of the taluka and of the district, has a population of about 20,000 and is situated on the southern bank of the Ghar Canal. The chief cultivation in the surrounding country is rice. Under the Lloyd Barrage Scheme the great new Central Rice Canal pours its water into the Ghar Canal just below the town, so that the part of the latter canal which runs along the border of the town is now a stagnant backwater. The River Indus flows about 7 miles to the east of the town. During the inundation season it overflows its banks, and the adjacent country, which is covered by a belt of forest, is flooded to a greater or less extent. A protective embankment, the nearest point of which is about 4 miles from the town, prevents the flood waters from actually reaching Larkana. The subsoil water level varies from 3 to 8 feet during the inundation period to 5 to 10 feet during the non-inundation period. Larvæ of *A. culicifacies* have been collected from the margins of the Ghar Canal during the period September to April each year since 1928. The breeding has been more profuse during the years 1932 and 1933, i.e., since the cutting off of the portion of the canal referred to above.

First survey (inter-epidemic).—In April 1927 the spleen rate was 20 per cent (250 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 71 per cent (1,613 observations), and the average enlarged spleen was 7.9 cm. The parasite rate was 24.9 per cent (245 observations, M. T., 56, B. T., 5). Crescents were observed in 5 cases.

MAHOTA is a village with about 1,000 inhabitants, situated 7 miles north-east of Larkana. The chief cultivation in the neighbourhood is rice, though there is some dry crop cultivation also. The Central Rice Canal flows within 400 yards from the village. In December 1932 the subsoil water level was 10 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 54 per cent (63 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 85 per cent (122 observations), and the average enlarged spleen was 6.0 cm.

CHUNNA is a village with about 150 inhabitants, situated half a mile to the north-east of Larkana, in the midst of date palm groves. In the surrounding country rice is the chief crop and sugar-cane is also grown. The Ghar Canal flows within about 400 yards of the edge of the village. In December 1932 the subsoil water level was 10 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 46 per cent (26 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 94 per cent (16 observations), and the average enlarged spleen was 5.4 cm.

BAROCH is a village with about 100 inhabitants, situated 2 miles north-east of Larkana, on the bank of the Ghar Canal. The chief cultivation is rice.

First survey (inter-epidemic).—In April 1927 the spleen rate was 36 per cent (11 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 87 per cent (8 observations), and the average enlarged spleen was 7.7 cm.

MASU HABB is a village with a population of about 750, situated 4 miles north of Larkana. To the north of the village rice is grown, whilst dry crops are cultivated to the south. In December 1932 the subsoil water level was 8 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 27 per cent (71 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 91 per cent (120 observations), and the average enlarged spleen was 6.3 cm. The parasite rate was 44 per cent (25 observations, M. T., 9, B.T., 2). Crescents were observed in 2 cases.

KHALID is a village with about 250 inhabitants, situated 6 miles east of Larkana, and surrounded by dry crops. Until 1929 the village was situated just outside the protection of the flood restriction embankment, but after the flood which occurred in that year the site was removed to one just within the embankment. The subsoil water level in December 1932 was 10 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 44 per cent (25 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 79 per cent (61 observations), and the average enlarged spleen was 8.0 cm.

AKIL is a village with a population of about 950, situated 8 miles east of Larkana, just inside the flood restriction embankment. The chief cultivation is rice. The subsoil water level in December 1932 was 7 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 27 per cent (59 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 55 per cent (42 observations), and the average enlarged spleen was 7.5 cm. The parasite rate was 19 per cent (26 observations, M. T., 5).

DHAMRAHA is a village with a population of about 1,000, situated 7 miles north of Larkana, in the midst of rice cultivation. The subsoil water level in December 1932 was 6 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 17 per cent (69 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 67 per cent (158 observations), and the average enlarged spleen was 7.0 cm. The parasite rate was 30 per cent (50 observations, M. T., 15). Crescents were observed in one case.

FATEHPUR is a village with about 400 inhabitants, situated 4 miles north of Larkana, in the midst of rice cultivation. In December 1932 the subsoil water level was 11 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 48 per cent (66 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 79 per cent (66 observations), and the average enlarged spleen was 6.7 cm. The parasite rate was 18 per cent (22 observations, M. T., 4).

SATARDINO MANGAN is a village with a population of about 300, situated 4 miles north of Larkana, in the midst of rice cultivation. A minor canal flows past the village. In December 1932 the subsoil water level was 7 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 69 per cent (39 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 90 per cent (40 observations), and the average enlarged spleen was 5.9 cm. The parasite rate was 28 per cent (25 observations, M. T., 7).

KANGA is a village with a population of about 900, situated 6 miles north-east of Larkana, in the midst of rice cultivation. The subsoil water level in December 1932 was 9 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 32 per cent (77 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 87 per cent (112 observations), and the average enlarged spleen was 7.5 cm. The parasite rate was 24 per cent (50 observations, M. T., 12). Crescents were observed in 2 cases.

BEORACHANDIA is a village with a population of about 800, situated 7 miles west of Larkana, in the midst of rice cultivation. In December 1932 the subsoil water level was 10 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 38 per cent (55 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 84 per cent (103 observations), and the average enlarged spleen was 8.5 cm.

NAZAR is a village with a population of about 550, situated 2 miles south of Larkana, in the midst of rice cultivation. In December 1932 the subsoil water level was 11 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 46 per cent (48 observations).

Second survey (post-epidemic).—In December 1932 the spleen rate was 96 per cent (53 observations), and the average enlarged spleen was 7·6 cm.

WALID is a village with about 1,200 inhabitants, situated on the bank of the Ghar Canal, 2 miles north of Larkana. Though it lies in a rice-growing area, there is no rice actually growing within 500 yards of the village. There is some date palm cultivation in the village. The subsoil water level in December 1932 was 7 feet.

First survey (inter-epidemic).—In November 1927 the spleen rate was 61 per cent (100 observations), and the average enlarged spleen was 7·9 cm. The parasite rate was 47 per cent (75 observations, M. T., 25, B. T., 8, Q., 2). Crescents were observed in 12 cases.

Second survey (inter-epidemic).—In July 1928 the spleen rate was 37 per cent (80 observations), and the average enlarged spleen was 8·8 cm. The parasite rate was 21 per cent (80 observations, M. T., 12, B. T., 2, Q., 3). Crescents were observed in 5 cases.

Third survey (height of epidemic).—In November 1929 the spleen rate was 91 per cent (93 observations), and the average enlarged spleen was 6·3 cm. The parasite rate was 74 per cent (39 observations, M. T., 29). Crescents were observed in one case.

Fourth survey (post-epidemic).—In October 1930 the spleen rate was 97 per cent (195 observations), and the average enlarged spleen was 8·2 cm. The parasite rate was 82 per cent (28 observations, M. T., 19, B. T., 4).

DODAI is a village with about 750 inhabitants, situated 2½ miles north of Larkana, in the midst of rice cultivation. There is also a grove of date palms in the village. The subsoil water level in December 1932 was 5 feet.

First survey (inter-epidemic).—In April 1927 the spleen rate was 71 per cent (41 observations).

Second survey (inter-epidemic).—In October 1927 the spleen rate was 54 per cent (33 observations), and the average enlarged spleen was 8·1 cm. The parasite rate was 12 per cent (33 observations, M. T., 3, B. T., 1).

Third survey (inter-epidemic).—In July 1928 the spleen rate was 53 per cent (43 observations), and the average enlarged spleen was 7·2 cm. The parasite rate was 21 per cent (43 observations, M. T., 8, Q., 1).

Fourth survey (height of epidemic).—In November 1929 the spleen rate was 88 per cent (76 observations), and the average enlarged spleen was 6·4 cm. The parasite rate was 74 per cent (50 observations, M. T., 34, B. T., 3). Crescents were observed in one case.

Fifth survey (post-epidemic).—In December 1932 the spleen rate was 99 per cent (90 observations), and the average enlarged spleen was 5·9 cm. The parasite rate was 34 per cent (50 observations, M. T., 17).

ANOPHELINE MOSQUITOES.

During the course of the survey carried out in Larkana District in November and December 1932, adult specimens of the following species of anophelines were collected in the various villages :—

<i>A. subpictus</i>	303
<i>A. culicifacies</i>	259
<i>A. pulcherrimus</i>	154
<i>A. stephensi</i>	5
<i>A. hyrcanus</i> var. <i>nigerrimus</i>	2
TOTAL					723

As has been shown in our previous papers, *A. culicifacies* is the principal, if not the sole, carrier of malaria in the province. The bionomics of the various species of anophelines occurring in the rice tract were discussed in detail in our paper dealing with the factors influencing the autumnal incidence of malaria in Larkana taluka (Covell and Baily, 1930). Dissections of *A. culicifacies* have been carried out in certain villages near Larkana since the year 1928, and the sporozoite rate by months over a period of 5 years is shown in Table XII. The rate is usually highest from mid-October to mid-December. In 1933 a specimen with sporozoites in the salivary gland was encountered in April for the first time; but as no dissections had been carried out in that month except in 1929 it is not possible to say whether or not this is a usual phenomenon.

DISCUSSION OF RESULTS.

EPIDEMIC MALARIA.

The observations recorded in the present paper were made in the great rice-growing area of Sind, a heavily irrigated tract with a subsoil water level varying in general from 5 to 12 feet, and most of the villages visited were surrounded by rice cultivation. The majority of the observations made prior to the epidemic of 1929 were carried out in Larkana taluka. The combined spleen rate for 13 localities in this taluka was 35 per cent, but this is certainly too low for the rice tract generally, because more than one-fourth of the children examined came from Larkana town. Even so, the figure is more than three times as great as that recorded in the dry crop area of Northern Sind at the same period.

The spleen rates recorded prior to the epidemic in other talukas of the rice tract were on the whole higher, e.g., Dokri 48 per cent, Nasirabad 74 per cent, Kambar 45 per cent and 37 per cent, Bher 77 per cent and 54 per cent. On the other hand, Nao Dero, which lies outside the rice tract, yielded figures of 22, 30, and 13 per cent, whilst Chowsool, where only dry crops had been cultivated for several years, had a figure of only 3 per cent.

On the whole, the rice tract of Sind may be described as a hyperendemic area, spleen rates usually ranging from the neighbourhood of 40 to 70 per cent before and after the annual malaria season respectively, whilst in certain areas where rice is only sparsely cultivated owing to lack of sufficient water, endemic malaria is moderate in amount.

THE EPIDEMIC OF 1929.

Larkana District felt the effects of this epidemic to a considerable extent, although the epidemic figures were not so high as in the Upper Sind Frontier and Sukkur Districts. The only localities in the district from which figures recorded during the actual epidemic period are available are the villages of Walid and Dodai, in Larkana taluka, and Kambar and Bher, in Kambar taluka. In July 1928 the spleen rates in these villages had been 37, 53, 37 and 54. In November 1929 the corresponding figures were 91, 88, 82 and 83. It is noteworthy that these high figures were reached in the rice tract during the height of the epidemic, whereas in those parts of Northern Sind where the inter-epidemic spleen rates had been very low, the rates at the height of the epidemic were only moderately raised, not reaching their maximum height of 80 to 90 per cent until about 8 months after the end of the actual epidemic period (Covell and Baily, 1932).

THE POST-EPIDEMIC PERIOD.

The observations recorded in Larkana District in November and December 1932 are of considerable interest. It might be expected that at this period, three years after the occurrence of the last regional epidemic, the spleen rates would have fallen to a considerable extent, and indeed that they would be not very much higher than they had been before the epidemic. This has occurred in three villages only of which we have records taken before the epidemic, namely Kambar, Bher and Nasirabad. In the case of the 13 localities in Larkana taluka referred to above, where the combined spleen rate prior to the epidemic was 35 per cent, the figure in December 1932 was 74 per cent. At Dokri, the figure had risen from 48 to 86, at Nao Dero from 13 to 65, at Chowsool from 3 to 79. Even allowing for the fact that the 1932 figures were recorded towards the end of the annual autumnal malaria season, they appear to be abnormally high. A reference to Table XI, which gives the results of blood examinations carried out during this period, shows that the parasite rate was generally high, and the proportion of crescent carriers in many cases considerable, suggesting that the transmission of malaria was being actively carried on, and that the malaria season was a severe one. This was corroborated by the statements of the villagers themselves.

**PROBABLE EFFECT OF THE LLOYD BARRAGE SCHEME ON
MALARIA IN LARKANA DISTRICT.**

Under the Lloyd Barrage Scheme the rice tract will be irrigated by the Central Rice Canal and its branches. The area to the east of this will be irrigated from the Dadu Perennial Canal, and the area to the north-west will receive perennial irrigation from the branches of the North-west Perennial Canal. As has been explained in our paper on Malaria in Dadu District (Covell and Baily, 1934), the rice area, which averaged 360,000 acres before the Barrage Scheme came into operation, will be increased eventually up to 580,000 acres, and this area will be surrounded on the north, north-west, south and east by perennial cultivation from the new canal system. Since the periodical lowering of the subsoil water level which occurred during the winter months in the rice area was presumably due, in part at least, to the movement of water through the subsoil from areas in which the level was high to areas

in which it was low, it seems possible that the new perennial irrigation which surrounds the rice area, may affect adversely the drainage capacity of the soil, and cause an upward trend in the level of the subsoil water, resulting eventually in water-logging (Hawes, 1932). Apart from this, a rise in the subsoil water level as the result of seepage from the new canals may also cause water-logging in certain areas. From whatever cause it may arise, any serious rise in the subsoil water level in this tract is likely to lead to an increase in malaria.

Observations made in June 1932 and June 1933 have shown that a marked rise occurred in the subsoil water level in large areas of the rice tract during the first year of the operation of the Barrage Scheme, and that the situation in regard to possible water-logging in these areas in the near future is a serious one.

But there are other ways in which the incidence of malaria may be affected by the operation of the Barrage Scheme. In certain places, existing canals are being used as the beds of the new canals. An instance of this is where the Central Rice Canal is led into the old Ghar Canal close to Larkana, resulting in the cutting off of part of the former canal and the creation of a species of backwater. We have observed in many parts of Sind that villages situated on the banks of old 'dhoros', or former river beds, are invariably highly malarious; and in places where loops of the old canals are cut off, it seems likely that malaria will be increased.

Another point to be considered is whether the introduction of so much water on to the land both earlier and later than was formerly the case will result in an increase in the length of the malaria transmission season, by increasing the relative humidity of the atmosphere.

The Barrage Scheme came into operation for the first time in the year 1932. As we have seen, all the evidence points to the fact that the incidence of malaria in the autumn of this year was unusually high throughout the area in which our observations were made. In the absence of any other cause, such as excessive rainfall or flooding, it seems very probable that the increase of malaria in this tract was actually due to the operation of the Barrage Scheme, through one or more of the factors which we have indicated above.

Whether the increase in malaria will be a permanent one or not will depend on the degree of success attained by the Irrigation Department in providing efficient drainage for the whole of the area on the right bank of the Indus which comes under the command of the Barrage Scheme.

SUMMARY.

1. An account is given of observations made in Larkana District during the period 1927-1932.
2. The 54 villages visited are almost all situated in the chief rice-growing area of Sind.
3. Malaria is hyperendemic in most of the villages in this tract.
4. Very high spleen rates were recorded during the height of the regional epidemic of 1929.
5. In the great majority of cases the spleen rates were extremely high in the autumn of 1932, when the tract experienced an unusually severe malaria season.

6. The probable effect of the operation of the Lloyd Barrage on the incidence of malaria in Larkana District is discussed. There has been a marked rise of subsoil water level in large areas of the rice tract since the Barrage Scheme commenced to operate in 1932, and the situation in regard to possible water-logging in the near future is a serious one. It is considered that the abnormally high incidence of malaria experienced in this area in 1932 was probably due to the effects of the operation of the Barrage Scheme.

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APPENDIX.

TABLE I.

Rainfall figures recorded at the headquarter towns of five talukas in Larkana District, for the months July–September, 1901–1932.

Years.	RATODERO.			KAMBAR.			WARAH.			LABDARYA.			LARKANA.			REMARKS.
	July.	Aug.	Sept.	July.	Aug.	Sept.	July.	Aug.	Sept.	July.	Aug.	Sept.	July.	Aug.	Sept.	
1901	0.31	0.26	0.30	0.02	..	0.60	0.01	..	1.13	Epidemic.
1902	..	0.79	0.85	..	0.39	1.07	..	0.16	0.96	..	1.80	0.20	0.02	0.93	1.88	
1903	4.54	1.33	1.39	3.16	5.78	
1904	
1905	0.45	..	0.04	0.18	0.23	..	0.06	0.11	..	0.43	0.40	..	0.03	
1906	..	2.71	0.36	..	0.85	1.24	0.79	0.04	..	1.33	0.85	
1907	..	1.85	..	0.54	1.81	..	0.20	1.70	..	0.10	2.68	..	0.11	2.27	..	
1908	1.16	1.34	..	0.62	0.05	..	0.20	0.50	..	0.46	0.10	..	1.43	0.30	..	
1909	0.10	0.05	..	0.19	0.18	0.31	0.26	
1910	3.33	2.78	3.56	3.23	
1911	
1912	0.22	0.86	..	0.43	0.45	..	0.02	0.75	..	0.55	0.34	..	0.04	1.47	0.05	
1913	0.83	1.83	..	1.71	0.90	..	1.67	1.07	..	2.34	0.53	..	1.41	1.52	..	
1914	1.29	..	0.07	2.24	3.05	0.54	..	0.01	1.07	
1915	0.11	0.12	0.14	0.10	

TABLE I—concl'd.

Years.	RATODERO.			KAMBAR.			WARAH.			LARDARYA.			LARKANA.			REMARKS.
	July.	Aug.	Sept.	July.	Aug.	Sept.	July.	Aug.	Sept.	July.	Aug.	Sept.	July.	Aug.	Sept.	
1916	..	1-55	..	0-05	0-70	0-08	0-11	4-85	3-46	3-21	..	Epidemic.
1917	..	9-89	6-73	..	6-29	7-23	0-12	3-85	7-65	..	7-02	9-26	..	7-85	8-27	
1918	0-08	0-83	
1919	1-33	0-46	1-05	0-72	2-18	0-08	..	
1920	0-70	0-28	0-32	0-04	Epidemic.
1921	0-03	0-03	..	0-22	0-25	0-10	0-42	1-15	..	0-37	0-58	..	0-51	0-02	0-13	
1922	0-01	..	0-75	..	0-02	
1923	0-25	2-66	..	0-02	1-25	1-09	..	0-01	0-82	..	0-25	0-79	..	
1924	2-78	0-49	..	2-96	0-89	..	3-20	0-17	..	1-64	1-75	Epidemic.
1925	0-52	1-03	..	1-15	3-86	..	1-98	0-76	..	0-42	1-94	..	1-81	0-94	..	
1926	..	0-22	0-90	0-63	..	0-68	2-32	..	0-51	0-36	..	0-11	0-10	
1927	0-31	0-50	..	0-60	0-75	..	0-86	0-14	..	0-32	..	0-03	1-23	0-30	..	
1928	0-20	0-60	Epidemic.
1929	5-16	1-94	..	6-63	1-40	..	4-50	2-26	..	6-75	2-75	..	8-65	2-67	..	
1930	1-32	1-68	2-60	0-85	1-15	
1931	0-70	0-10	0-10	..	0-17	1-53	0-20	0-70	..	
1932	2-30	1-62	..	3-80	1-43	..	3-40	1-63	..	3-68	3-26	..	0-27	2-74	..	

N.B.—Figures for Mirokhan taluka are not available.

TABLE II.

Maximum and minimum temperatures recorded at Larkana town during the months of January, May, July and November, 1913-1922.

Years.	JANUARY.		MAY.		JULY.		NOVEMBER.	
	Max. °F.	Min. °F.	Max. °F.	Min. °F.	Max. °F.	Min. °F.	Max. °F.	Min. °F.
1913	76	50	114	85	107	86	85	51
1914	74	52	115	81	118	80	86	59
1915	74	51	112	86	112	87	90	56
1916	75	50	108	90	108	86	84	49
1917	76	48	104	79	115	80	91	60
1918	67	43	111	78	111	70	88	58
1919	70	40	103	80	110	90	78	58
1920	68	49	100	75	111	87	91	52
1921	68	40	112	86	106	80	88	60
1922	68	46	112	81	104	83	85	60

TABLE III.

Epidemic figures of six talukas of Larkana District for the period 1901-1932.

Years.	Ratodero.	Kambar.	Warah.	Labdarya.	Larkana.	Mirokhan.*	REMARKS.
1901	7'36	3'37	1'93	4'30	4'66	..	Epidemic.
1902	5'16	3'37	3'32	2'65	2'91	..	
1903	1'97	2'21	2'34	2'36	1'90	..	
1904	11'20	1'32	1'45	1'95	1'13	..	
1905	2'85	2'23	2'25	2'60	2'33	..	
1906	3'63	2'40	2'02	5'32	2'63	..	
1907	1'64	1'49	1'95	2'87	1'28	..	
1908	1'45	1'52	1'09	3'35	1'31	..	
1909	1'98	1'01	0'98	2'18	1'61	..	
1910	1'60	1'60	0'84	2'56	1'98	..	
1911	0'80	1'95	0'95	1'48	1'98	1'64	
1912	2'09	3'31	1'75	1'96	3'30	2'95	
1913	3'40	2'53	1'98	2'61	3'50	4'40	
1914	1'78	2'73	2'68	2'12	2'40	1'97	
1915	1'16	1'93	1'39	1'46	2'01	2'12	Epidemic.
1916	2'14	5'56	2'72	3'90	4'00	3'89	
1917	8'45	9'15	4'93	9'00	10'70	9'95	
1918	
1919	
1920	0'96	0'98	0'93	1'15	1'43	1'35	
1921	1'83	1'80	1'03	1'45	1'95	1'78	
1922	1'82	1'57	1'03	1'61	2'21	1'95	
1923	0'77	1'25	0'81	0'75	1'43	1'66	
1924	1'60	2'50	1'75	1'78	2'37	2'04	
1925	0'92	1'84	1'48	6'75	0'83	1'88	
1926	1'19	1'60	2'12	1'26	1'45	1'42	
1927	1'47	2'03	2'24	6'20	1'57	2'24	
1928	1'22	1'86	1'37	1'06	1'92	2'09	Epidemic.
1929	5'45	3'23	3'07	5'50	3'18	6'60	
1930	1'77	1'09	1'23	1'81	1'62	2'07	
1931	1'40	1'10	1'20	1'90	2'30	2'20	
1932	1'60	1'80	1'30	1'30	3'50	2'30	

* Mirokhan taluka was constituted in 1911.

TABLE IV.

Results of spleen examinations in Ratodero taluka.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
INTER-EPIDEMIC PERIOD.					
Nao Dero ..	iv. 27	146	33	22.5	..
Nao Dero ..	xi. 27	100	30	30.0	7.9
Nao Dero ..	vii. 28	90	12	13.3	7.4
POST-EPIDEMIC PERIOD.					
Nao Dero ..	xi. 32	145	94	64.8	6.6
Ratodero ..	xi. 32	296	167	56.6	7.7
Pir Jo Got ..	xi. 32	100	68	68.0	5.9
Panjo Dero ..	xi. 32	88	72	81.8	7.5
Khan Wah ..	xi. 32	73	46	63.0	7.2
Pir Baksh Bhuto ..	xi. 32	67	44	65.7	7.7
Masu Dero ..	xi. 32	40	27	67.5	8.0
Ghari Khuda Baksh ..	xi. 32	55	29	52.7	6.4
Bungal Dero ..	xi. 32	276	164	59.4	6.5

TABLE V.

Results of spleen examinations in Miro Khan taluka.

INTER-EPIDEMIC PERIOD.					
Chowsool ..	iv. 27	33	1	3.2	..
POST-EPIDEMIC PERIOD.					
Chowsool ..	iii. 31	43	35	81.1	7.0
Chowsool ..	xi. 32	47	37	78.7	6.0
Miro Khan ..	xi. 32	80	40	50.0	8.4
Joya ..	xi. 32	65	40	61.5	6.1
Langhari ..	xi. 32	18	8	44.4	7.9
Tharo Wadho ..	xi. 32	40	31	77.5	6.7
Sujawal ..	xi. 32	102	61	60.0	6.8
Banglow Mahbab ..	xi. 32	58	40	69.0	7.0
Buthi ..	xi. 32	58	42	72.4	6.2
Bahram Hethian ..	xi. 32	30	18	60.0	8.4

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE VI.

Results of spleen examinations in Kambar taluka.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
INTER-EPIDEMIC PERIOD					
Kambar	iv. 27	126	56	45.2	..
Kambar	xi. 27	112	51	45.5	8.5
Kambar	vii. 28	100	37	37.0	5.6
Bher	i. 28	83	64	77.1	7.1
Bher	vii. 28	50	27	54.0	7.0
EPIDEMIC PERIOD.					
Kambar	xi. 29	147	121	82.3	7.2
Bher	xi. 29	82	69	84.1	7.4
POST-EPIDEMIC PERIOD.					
Kambar	xi. 32	200	97	48.5	7.6
Bher	xi. 32	110	63	57.3	7.4
Ghatahar	xi. 32	115	68	59.1	7.4
Hani	xi. 32	47	31	66.0	7.0
Mena	xi. 32	83	48	57.8	7.7
Hulia	xi. 32	45	11	24.4	8.4
Got Nur Mohd. Khan	xi. 32	40	24	60.0	7.4
Mehan Ji Khai ..	xi. 32	67	48	71.6	7.0
Daphar	xi. 32	34	20	58.8	8.1

TABLE VII.

Results of spleen examinations in Warah taluka.

POST-EPIDEMIC PERIOD.					
Warah	xi. 32	185	147	79.4	7.0
Nasirabad † ..	xi. 32	222	134	60.3	6.9
Panhwar and Muradi	xi. 32	30	15	50.0	7.5
Lakha	xi. 32	72	43	59.7	7.5
Ghazi Khuhawar ..	xi. 32	252	101	40.0	8.0
Jani Jo Band ..	xi. 32	108	63	58.3	7.5
Haitam Saho ..	xi. 32	55	16	29.0	8.1
Wagan	xi. 32	125	93	74.4	8.1

* Measurement in centimetres from apex of spleen to umbilicus.

† The spleen rate in this village in January 1928 (inter-epidemic period) was 74 per cent.

TABLE VIII.
Results of spleen examinations in Labdarya taluka.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
INTER-EPIDEMIC PERIOD.					
Dokri	iv. 27	56	27	48.2	..
POST-EPIDEMIC PERIOD.					
Dokri	xi. 32	151	130	86.0	6.2
Badeh	xi. 32	300	163	54.3	7.1
Darra	xi. 32	36	26	72.2	6.8
Gerelo	xi. 32	220	149	67.7	6.4
Tharecha ..	xi. 32	58	49	84.5	7.5
Mad Bahu ..	xi. 32	105	87	82.9	7.0

TABLE IX.
Results of spleen examinations made in Larkana taluka.

INTER-EPIDEMIC PERIOD.					
Larkana	iv. 27	250	51	20.4	..
Mahota	iv. 27	63	34	54.0	..
Baroch	iv. 27	11	4	36.4	..
Chunna	iv. 27	26	12	46.2	..
Masu Habb ..	iv. 27	71	19	26.7	..
Khalid	iv. 27	25	11	44.0	..
Akil	iv. 27	59	16	27.1	..
Dhamraha ..	iv. 27	69	12	17.4	..
Fatehpur ..	iv. 27	66	32	48.5	..
Satardino ..	iv. 27	39	27	69.2	..
Kanga	iv. 27	77	25	32.4	..
Nasar	iv. 27	48	22	46.0	..
Beorachandia ..	iv. 27	55	21	38.2	..
TOTAL	iv. 27	859	286	34.6	..
POST-EPIDEMIC PERIOD.					
Larkana	xii. 32	1,613	1,136	71.3	7.9
Mahota	xii. 32	122	104	85.2	6.0
Baroch	xii. 32	8	7	87.5	7.7
Chunna	xii. 32	16	15	93.8	5.4
Masu Habb ..	xii. 32	120	109	90.8	6.3
Khalid	xii. 32	61	48	78.7	8.0
Akil	xii. 32	42	23	54.8	7.5
Dhamraha ..	xii. 32	158	106	67.0	7.0
Fatehpur ..	xii. 32	66	52	78.8	6.7
Satardino ..	xii. 32	40	36	90.0	5.9
Kanga	xii. 32	112	97	86.6	7.5
Nasar	xii. 32	53	51	96.2	7.6
Beorachandia ..	xii. 32	103	87	84.5	8.5
TOTAL	xii. 32	2,514	1,871	74.4	7.9

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE X.

Results of repeated spleen examinations made in 5 villages of Larkana District, 1927 to 1932.

Locality.	Date.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
INTER-EPIDEMIC PERIOD.					
Nao Dero	iv. 27	146	33	22.5	..
Nao Dero	xi. 27	100	30	30.0	7.9
Nao Dero	vii. 28	90	12	13.3	7.4
Kambar	iv. 27	126	56	45.2	..
Kambar	xi. 27	112	51	45.5	8.5
Kambar	vii. 28	100	37	37.0	5.6
Bher	i. 28	83	64	77.1	7.1
Bher	vii. 28	50	27	54.0	7.0
Dodai	iv. 27	41	29	70.7	..
Dodai	x. 27	33	18	54.5	8.1
Dodai	vii. 28	43	23	53.5	7.2
Walid	xi. 27	100	61	61.0	7.9
Walid	vii. 28	80	30	37.5	8.8
EPIDEMIC PERIOD.					
Kambar	xi. 29	147	121	82.3	7.2
Bher	xi. 29	82	69	84.1	7.4
Dodai	xi. 29	76	67	88.2	6.4
Walid	xi. 29	93	85	91.4	6.3
POST-EPIDEMIC PERIOD.					
Nao Dero	xi. 32	145	94	64.8	6.6
Kambar	xi. 32	200	97	48.5	7.6
Bher	xi. 32	110	63	57.3	7.4
Dodai	xii. 32	90	89	98.8	5.9
Walid	x. 30	195	189	97.0	8.2

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE XI.

Results of blood examinations made in Larkana District.

Locality.	Date.	Number examined.	Number with parasites.	Parasite rate.	M. T. infection rate.	Crescent rate.	B. T. infection rate.	Q. infection rate.
INTER-EPIDEMIC PERIOD.								
Dodai	x. 27	33	4	12.1	9.1	0.0	3.0	0.0
Nao Dero	xi. 27	90	13	14.4	7.7	5.5	6.6	0.0
Walid	xi. 27	75	35	46.6	33.3	16.0	10.6	2.6
Kambar	xi. 27	112	35	31.2	14.3	4.4	17.0	0.0
Bher	i. 28	83	24	28.8	16.8	12.0	0.0	12.0

TABLE XI—concl'd.

Locality.	Date.	Number exa- mined.	Number with para- sites.	Parasite rate.	M. T. infection rate.	Cres- cent rate.	B. T. infection rate.	Q. infection rate.
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INTER-EPIDEMIC PERIOD—concl'd.

Kambar	..	vii. 28	100	35	35'0	31'0	10'0	4'0	1'0
Dodai	..	vii. 28	43	9	20'9	18'6	0'0	0'0	2'3
Nao Dero	..	vii. 28	90	9	10'0	7'7	1'1	2'2	0'0
Walid	..	vii. 28	80	17	21'2	15'0	6'2	2'5	3'7
Bher	..	vii. 28	50	5	10'0	10'0	0'0	0'0	0'0

EPIDEMIC PERIOD.

Walid	..	xi. 29	39	29	74'3	74'3	2'6	0'0	0'0
Bher	..	xi. 29	50	40	80'0	74'0	8'0	6'0	0'0
Dodai	..	xi. 29	50	37	74'0	68'0	2'0	6'0	0'0
Kambar	..	xi. 29	50	42	84'0	84'0	4'0	0'0	0'0

POST-EPIDEMIC PERIOD.

Walid	..	x. 30	28	23	82'2	67'8	0'0	14'3	0'0
Dodai	..	xii. 32	50	17	34'0	34'0	0'0	0'0	0'0
Nao Dero	..	xi. 32	20	7	35'0	35'0	15'0	0'0	0'0
Ratodero	..	xi. 32	100	29	29'0	25'0	7'0	6'0	0'0
Pir Jo Got	..	xi. 32	20	8	40'0	40'0	30'0	0'0	0'0
Panjoo Dero	..	xi. 32	10	4	40'0	40'0	10'0	0'0	0'0
Khan Wah	..	xi. 32	25	11	44'0	40'0	12'0	4'0	0'0
Masu Dero	..	xi. 32	25	7	28'0	28'0	8'0	0'0	0'0
Ghari K h u d a	..	xi. 32	25	13	52'0	48'0	24'0	4'0	0'0
Baksh.									
Joya	..	xi. 32	25	6	24'0	14'0	12'0	0'0	0'0
Sujawal	..	xi. 32	25	7	28'0	28'0	0'0	0'0	0'0
Chowsool	..	xi. 32	19	6	31'6	31'6	5'2	0'0	0'0
Mirokhan	..	xi. 32	50	9	18'0	18'0	2'0	0'0	0'0
Ghatahar	..	xi. 32	43	11	25'6	25'6	2'3	0'0	0'0
Kambar	..	xi. 32	100	9	9'0	9'0	4'0	0'0	0'0
Nasirabad	..	xi. 32	50	13	26'0	26'0	2'0	0'0	0'0
Ghazi Khuhawar	..	xi. 32	34	8	23'6	23'6	3'0	0'0	0'0
Wagan	..	xi. 32	61	8	13'1	13'1	0'0	0'0	0'0
Dokri	..	xi. 32	80	25	31'2	31'2	7'5	0'0	0'0
Badeh	..	xi. 32	50	13	26'0	26'0	4'0	0'0	0'0
Masu Habb	..	xii. 32	25	11	44'0	36'0	8'0	8'0	0'0
Akil	..	xii. 32	26	5	19'2	19'2	0'0	0'0	0'0
Dhamraha	..	xii. 32	50	15	30'0	30'0	2'0	0'0	0'0
Fatehpur	..	xii. 32	22	4	18'2	18'2	0'0	0'0	0'0
Satardino	..	xii. 32	25	7	28'0	28'0	0'0	0'0	0'0
Kanga	..	xii. 32	50	12	24'0	24'0	4'0	0'0	0'0
Larkana	..	xii. 32	245	61	24'9	22'8	2'0	2'0	0'0

A MOSQUITO-FLIGHT EXPERIMENT.*

BY

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[27th February, 1934]

THE following is an account of an experiment carried out at Vizagapatam Harbour, with the object of finding out whether cases of malaria occurring on one side of the entrance channel could be caused by mosquitoes breeding on the opposite side.

EXPERIMENT.

COLLECTION OF MOSQUITOES.

About 1,300 mosquitoes, both anophelines and culicines, some bred out in the laboratory and some caught in nature, were collected in a cage. These were given blood feeds successively for 2 days prior to the date of their release at Lova Gardens.

PREPARATION OF MOSQUITOES.

All the mosquitoes were transferred to a small cage from the bigger one in the morning on which they were released. The number that was transferred to the small cage was only 400 as the rest had died. All these mosquitoes were sprayed with methylene blue (1 in 10,000). Spraying was done with a hair lotion bottle fitted with a pump.

* This article was accompanied by a map and several photographs. It is regretted that these cannot be reproduced here, but they are available for reference in the library of the *Malaria Survey of India, Kasauli.*

RELEASE OF MOSQUITOES.

The small cage containing the stained mosquitoes was taken to the Lova Gardens with much care and the mosquitoes were released at a bungalow situated about 900 yards distant from the Port Office.

RE-CATCH OF MOSQUITOES.

Continuously for about three weeks catches were made on the northern side of the entrance channel. For one or two days subsequent to the release there were heavy rains. The mosquitoes that were caught every day were chloroformed and their wings examined under a microscope. On 3-ix-1933 one *Culex*, on 4-ix-1933 one *A. pallidus*, and on 16-ix-1933 one *A. subpictus* which were caught in the 'Dredger Khalasis Quarters' which are very near to the Engineer-in-Chief's Office, in the 'Port Office', and again in the 'Dredger Khalasis Quarters' respectively, showed blue colour in their wings under the microscope.

Details of experiment and catches.

Date.	Number of stained mosquitoes released at Lova Gardens.	Date and number of mosquitoes caught.	Place where caught and distance from the Lova Gardens.	Species.
27-viii-1933	310	On 3-ix-1933 one blue-stained mosquito.	Dredger Khalasis Quarters. About 800 yards.	<i>Culex</i> .
30-viii-1933	40	On 4-ix-1933 one blue-stained mosquito. On 16-ix-1933 one blue-stained mosquito.	Port Office. About 900 yards. Dredger Khalasis Quarters. About 800 yards.	<i>A. pallidus</i> . <i>A. subpictus</i> .
28-x-1933	50	Nil.	Nil.	Nil.

From the above experiment it has been definitely proved that mosquitoes, both anophelines and culicines, can easily fly over the entrance channel and feed upon the people residing on the southern side of the Darga Hill—a place where our workmen are living.

INCIDENCE OF MALARIA IN THE NORTHERN SIDE OF THE LOVA GARDENS.

The following statement shows the incidence of malaria amongst the Harbour employees attending the Harbour Dispensary from the Fort Ward, i.e., northern side of the Darga Hill—an untreated area—compared with the incidence of malaria amongst the Harbour employees attending the Harbour Dispensary, living on the southern side of the Darga Hill, i.e., on the northern side of the Lova Gardens—a treated area, and an area which was once evacuated by the inhabitants some decades back.

Average number of malaria cases attending the Harbour Dispensary.

Year.	Amongst the Harbour employees living in the Fort Ward on the northern side of the Darga Hill— an untreated area.	Amongst the people residing on the southern side of the Darga Hill— a treated area.*
1930	5.50	0.08
1931	6.25	0.03
1932	4.91	0.50
1933	3.91	0.83

Note.—The population of Harbour employees living on both the sides of the Darga Hill is almost the same according to the census taken by me in the year 1930.

* This is the place where a village by name Zalarupeta once existed the inhabitants of which were compelled to evacuate it on account of the high incidence of malaria.

CONCLUSION.

I believe that it is absolutely essential to carry on temporary measures like oiling and Paris-greening in the valley of 'Lova Gardens'. The valley is a potential breeding place for *A. culicifacies*, *A. fluviatilis* and *A. stephensi*. Interesting species like *A. tessellatus*, *A. jeyporiensis*, *A. karwari* and *A. varuna* are occasionally found here.

I am thankful to Lieut.-Col. F. J. Anderson, M.C., M.B.B.S., F.R.C.S., I.M.S., Ex-Chief Medical Officer, Vizagapatam Harbour, for having given me the proper stimulus and guidance for carrying on this experiment; and I am also highly thankful and deeply grateful to W. C. Ash, B.Sc., M.I.C.E., A.M.I.M.E., M.I.E., Engineer-in-Chief, and to Major J. A. W. Ebdon, M.D., M.S., F.R.C.S., I.M.S., Chief Medical Officer, Vizagapatam Harbour, for having permitted me to publish this and for providing me with all the facilities.

SOME NOTES ON THE IDENTIFICATION OF SOME ANOPHELINE LARVÆ BY MACROSCOPIC METHODS.

BY

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[28th February, 1934.]

IN making a survey of anopheline larvæ as a preliminary to the adoption of anti-malarial measures, a considerable amount of assistance can be obtained by closely observing the naked-eye appearances of larvæ which have been freshly caught. These appearances are only of value in larvæ which have been recently collected, as the natural appearances of larvæ are lost in specimens which are kept in the laboratory for more than two or three days. Identification requires only normal eyesight and minute attention to detail. Careful study for a short time will enable any intelligent observer to pick out larvæ with a considerable degree of accuracy in the field. It has been found that even coolie catchers become very expert after a short training and can identify the commoner larvæ with the naked eye.

Anopheline larvæ can be identified by their coloration, shape, size of the body, and in some instances by special markings. The larvæ for identification must be mature. There is no fixed colour in immature larvæ as their colour develops after their skin has been shed for the second instar. In some cases larvæ change their colour in different types of breeding ground and acquire colour closely resembling the water in which they are breeding. This is probably a defensive mechanism, but is sometimes due to deposition of organic material on the larvæ; sometimes to the changes induced by Ecto-parasites such as, *Vorticella* or members of the *Hydrachnidæ*; and, in the colder months, probably due to food deficiency.

METHODS OF IDENTIFICATION.

Place the living larvæ in a flat white porcelain dish so that they will lie on the surface of the water in the dish. Without disturbing or moving the dish, look quietly for the distinguishing features.

At the time of feeding, larvæ turn the head upside down and begin to move the feeding brushes and, where this occurs, the head should be turned over gently with the help of a dissecting needle.

MACROSCOPIC APPEARANCES.

The following are some of the macroscopic appearances of the commoner anopheline larvæ found in Assam :—

A. minimus.

A small thin larva of a light brownish colour (sometimes blackish).

Head.—Frontal portion of head is slightly darker than the occipital portion.

Thorax.—Broad with a light bluish colour on the anterior part of the thorax.

Abdomen.—There is a black line down the middle of the dorsum. The sides of the abdomen are slightly lower than the middle as the dorsum of the abdomen is slightly convex. The sides of the abdomen are *straight*.

A. aconitus.

A small thin, brownish larva (sometimes greenish black).

Head.—Frontal portion is dark (darker than in *A. minimus*).

Thorax.—Broad with brownish colour on the anterior part.

Abdomen.—The dorsum of the abdomen is slightly concave from side to side, the edges being a little higher than the middle. There is a prominent longitudinal black line extending from the thorax to the abdominal segment. The sides of the abdomen appear straight, but not so straight as in *A. minimus*. *A. minimus* can be differentiated from *A. aconitus* by the slight convexity in *A. minimus* and the slight concavity in *A. aconitus*.

A. jeyporiensis.

A small larva greenish in colour.

Head.—As in *A. aconitus*, frontal portion is dark.

Thorax.—Not so broad as in *A. aconitus* or *A. minimus* and the anterior portion is not darker as in *A. aconitus*.

Abdomen.—Is slightly concave (and there is no black line down the middle of the abdomen, as in *A. minimus*; the sides are slightly more straight than in *A. aconitus*).

A. hyrcanus.

The larva is large, robust and stout (not so stout as *A. barbirostris*). Greenish brown in colour, sometimes black with light silvery spots on the thorax and abdomen. The sides of the abdomen are not straight but show minute serrations. In young larvæ white bands on the neck and the body are always present. There are usually four or five white bands in all on the abdomen and neck.

A. barbirostris.

A big, strong and stout larva, dark brown in colour, sometimes almost black, with scattered silvery spots on the thorax and abdomen. The body is slightly thicker than *A. hyrcanus*. Young larvæ show no white bands on the neck and abdomen.

A. umbrosus.

Big, strong and stout in build, dark brown in colour, with prominent black spots on every abdominal segment. The sides of the abdomen appear quite straight owing to the absence of palmate hairs. The larvæ appear rather thick from the dorsal to the ventral surface.

A. gigas.

A big, stout and strong larva, brown in colour.

Head.—The occipital portion is slightly higher than the frontal.

A. kochi.

A fairly large larva, light brownish in colour. The full grown larva is larger than either *A. hyrcanus* or *A. barbirostris* larva.

Head.—Slightly smaller than the thorax.

Thorax.—The thorax is broad, with a prominent white spot on the anterior portion.

Abdomen.—The dorsal surface of the abdomen is slightly concave. The sides of the abdomen are thin and transparent.

A. tessellatus.

A small larva (smaller than *A. kochi*), greenish in colour.

Thorax.—Broad (not so broad as in *A. kochi*), with a prominent white spot on the anterior portion.

Abdomen.—The dorsal surface of the abdomen is slightly concave. The sides of the abdomen *are not differentiated as in A. kochi*.

A. leucosphyrus.

A fairly large larva (larger than either *A. kochi* or *A. tessellatus*), greenish brown in colour.

Thorax.—As in *A. kochi* with a white spot on the anterior part.

Abdomen.—The dorsal surface of the abdomen is slightly concave. The sides of the abdomen are not differentiated.

A. philippinensis.

A fairly large larva, greenish black in colour with a prominent white band on the neck and with white spots on the abdomen. Sometimes the larva is greenish brown in colour without any white bands on the neck. The dorsum of the abdomen is flat and the edges of the abdomen show *faint serrations*.

A. annularis.

A fairly large larva of the same size as *A. philippinensis*, greenish in colour (sometimes greenish black), without any white bands on the neck and abdomen. The edges of the abdomen show faint serrations.

A. karwari.

A fairly large larva, brownish in colour.

Head.—Frontal portion is blackish.

Abdomen.—The dorsum of the abdomen is slightly convex and the sides of the abdomen are not serrated.

A. maculatus.

A fairly large larva, light brownish in colour (lighter than *A. karwari*). The full grown larva is slightly larger than *A. karwari*.

Head.—Frontal portion is not so dark as in *A. karwari*.

Abdomen.—The dorsum of the abdomen is slightly convex and the sides of the abdomen are not serrated.

A. culicifacies.

A fairly large larva, light greenish in colour. This larva sometimes closely resembles *A. vagus* when it breeds in pools or in muddy water. It is somewhat difficult to differentiate this species from *A. vagus*. The dorsum of the abdomen is flat and there are no spots or markings on the thorax or abdomen.

A. vagus.

A fairly large larva, grey in colour. The dorsal surface is quite plain with sometimes small white and black spots on the body. The dorsal surface of the abdomen is flat.

A. aitkeni.

A very thin larva, light brownish in colour.

Thorax.—The thorax is broad.

Abdomen.—The dorsal surface of the abdomen is markedly concave.

Head.—The frontal portion is slightly brown and darker than the occipital, but not so dark as in *A. aconitus*.

Sometimes *A. aconitus* may be confused with *A. aitkeni* but the latter can be differentiated by its body which is much thinner than *A. aconitus*.

A. ramsayi.

Small, short and delicate larva, deep bluish in colour. The body of this larva is slightly thicker than *A. minimus* or *A. aconitus* larva. The deep bluish colour is its characteristic.

The following table gives the characteristics of each species clearly.

Table showing the principal points of identification of some anopheline larvae by macroscopical methods.

Species.	Colour.	Size.	Head.	Thorax.	Abdomen.	Sides.
<i>A. aconitus</i>	Brownish (sometimes black).	Small and thin	Frontal part darkest than <i>A. minimus</i> .	Broad, brownish in colour.	Prominent black line down the middle of the dorsum.	Straight but not so straight as in <i>A. minimus</i> .
<i>A. aikeni</i>	Light brownish	Very thin	Frontal portion slightly brown and darker than the occipital.	Broad	Dorsal surface convex.
<i>A. annularis</i>	Greenish (sometimes black).	Same as <i>A. philippinensis</i>	With no white spots.	Faint serrations.
<i>A. barbirostris</i>	Dark brown (sometimes almost blue).	Big, strong and stout in build, thicker than <i>A. hyrcanus</i>	With scattered silvery spots.	With scattered silvery spots.
<i>A. culicifacies</i>	Light greenish	Fairly large	With no spots	Dorsum flat.
<i>A. gigas</i>	Brown	Big, strong and stout, bigger than <i>A. hyrcanus</i> and <i>A. barbirostris</i> .	Occipital portion slightly higher.	Large
<i>A. hyrcanus</i>	Greenish brown (sometimes blackish).	Large, robust and stout.	With light silvery spots.	With light silvery spots.	Minute serrations.
<i>A. jeyporiensis</i>	Greenish	Small	Frontal portion dark, as in <i>A. aconitus</i> .	Not so broad as in <i>A. aconitus</i> or <i>A. minimus</i> , and the anterior part is not so dark as in <i>A. aconitus</i> .	Slightly concave (and there is no black line as in <i>A. minimus</i>).	Slightly more straight than <i>A. aconitus</i> .
<i>A. karwari</i>	Brownish	Fairly large	Frontal portion blackish.	Dorsum slightly convex.	Not serrated.

Table showing the principal points of identification of some anopheline larvae by macroscopical methods—concl

Species.	Colour.	Size.	Head.	Thorax.	Abdomen.	Sides.
<i>A. kochi</i>	Light brownish	Fairly large	Slightly smaller than thorax.	With prominent white spots on the anterior portion.	Dorsal surface slightly concave.	Thin and transparent.
<i>A. leucosphyrus</i>	Greenish brown	Larger than <i>A. kochi</i> and <i>A. tessellatus</i>	As in <i>A. kochi</i> .	Nothing distinctive.	Not differentiated.
<i>A. maculatus</i>	Light brownish (lighter than <i>A. karwar</i>).	Fairly large	Not so dark as in <i>A. karwar</i>	Dorsum slightly convex.	Not serrated.
<i>A. minimus</i>	Light brownish (sometimes blackish).	Small and thin	Frontal part darker than the occipital.	Broad with light bluish colour on the anterior part of the thorax.	Black line down the middle of dorsum.	Straight.
<i>A. philippinensis</i>	Greenish black (sometimes greenish brown).	Fairly large	With white spots.	Faint serrations.
<i>A. ramseyi</i>	Deep bluish	Small, short and delicate. Body slightly thicker than <i>A. minimus</i> and <i>A. acornatus</i>
<i>A. tessellatus</i>	Greenish	Smaller than <i>A. kochi</i>	Broad (not so broad as in <i>A. kochi</i>) with prominent white spots on the anterior portion.	Dorsal surface slightly concave.	Not differentiated as in <i>A. kochi</i> .
<i>A. umbrosus</i>	Dark brown	Big, strong and stout.	Prominent black spots on every segment.	Perfectly straight.
<i>A. vagus</i>	Grey	Fairly large	With white and black spots.	Dorsum flat, with white and black spots.

No attempt has been made in this paper to use entomological terms, or to give more than the simplest anatomical description. The paper is not meant to be an absolute guide, but merely a synopsis of the naked-eye appearances of each species which require only ordinary eyesight and no particular skill to discern. It is possible that the coloration, etc., may, in other parts of India, differ according to climate, food supplies and temperature.

The object of this paper is to stimulate an interest in field work, and to encourage observation.

The findings should always be checked by microscopic observation in the laboratory.

Several Medical Officers in the tea districts of Assam have expressed a desire to have these notes in printed form as a guide to larval identification in the field.

I have to thank Dr. L. R. Dey, Laboratory Assistant, Central Laboratory, Cinnamara, for much valuable assistance in preparing these notes.

STUDIES OF MALARIAL PIGMENT (HÆMOZOIN).

Part III.

FURTHER RESEARCHES INTO THE ACTION OF SOLVENTS, AND THE RESULTS OF OBSERVATIONS ON THE ACTION OF OXIDISING AND REDUCING AGENTS, ON OPTICAL PROPERTIES, AND ON CRYSTALLISATION.

BY

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(Indian Research Fund Association.)

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THE results of investigations into the action of different solvents, the absorption spectra seen in solutions, and the reactions of hæmozoin to tests for iron, have been recorded in two previous papers (Sinton and Ghosh, 1934; Ghosh and Sinton, 1934). These researches suggest very strongly that the hæmozoin formed by the malarial parasites of monkeys is probably identical with hæmatin. The results of various other experiments carried out to obtain further confirmation of these observations are recorded in the present paper.

I. RATES OF SOLUTION OF PURIFIED HÆMOZOIN AND HÆMATIN IN DIFFERENT SOLVENTS.

It was noted previously (Sinton and Ghosh, 1934) that, under the conditions of our experiments, the rate of solution of hæmatin was more rapid than that of hæmozoin with many of the solvents tested. Several different factors were suggested as possible explanations for this seeming discrepancy between two substances which appeared to be identical. Further experimentation has now been undertaken in an attempt to shed more light on the subject.

These differences in the rates of solution appeared to be due mainly to the fact that the hæmozoin granules were still surrounded by a protein covering, formed of the remains of the parasites. If the pigment were separated from this covering, it should then dissolve at almost the same rate as did hæmatin, if the two substances be identical and the explanations correct.

The pigment was extracted from a parasite suspension* by treatment with N/2 Na_2CO_3 aqueous solution, and any parasitic remains were separated by centrifugalisation. The pigment was reprecipitated from the supernatant fluid by the addition of oxalic acid. The oxalic acid was afterwards removed by repeated washing with distilled water and centrifugalisation.

The rate of solution of this purified hæmozoin was then compared with that of hæmatin in a few of the solvents which had previously given the most divergent results. The results obtained are shown in Table I.

TABLE I.

Solvent.	RATE OF SOLUTION.	
	Hæmozoin.	Hæmatin.
Pyridine	++	++
Quinine (0·4 per cent) in CHCl_3	+	+
Glacial acetic acid	++	++
N/10 KOH (aqueous solution)	+++	+++
N/2 Na_2CO_3 (aqueous solution)	++	++

It is seen from this table that the two pigments were found to have practically the same rates of solution in the various solvents tried. While in

*This suspension was prepared by the method described in detail by Sinton and Ghosh (1934).

the previous experiments (Sinton and Ghosh, 1934) pyridine dissolved hæmatin in about 1 hour, it took about 40 hours to dissolve malarial pigment, when this was still enclosed in the parasitic remains. In the present experiments both pigments dissolved within one hour. Similar results were obtained with quinine-chloroform solution and the other solvents tested.

SUMMARY.

It was found in earlier experiments that the rate of solution of purified hæmatin was more rapid in certain solvents than that of malarial pigment, which was still contained inside the remains of parasites. When, however, purified hæmozoin was used no differences between the rates of solution of the two pigments could be detected.

These results support the view that hæmozoin is identical with hæmatin. They also show that the variations in the rates of solution recorded in our previous work were probably due to the causes suggested (Sinton and Ghosh, 1934).

II. THE ACTION OF OXIDISING AND REDUCING AGENTS ON HÆMOZOIN AND ON HÆMATIN.

(i) OXIDISING AGENTS.

(a) Hydrogen peroxide.

Brown (1911a) reports that hydrogen peroxide solutions decolorise both hæmatin and hæmozoin. He found that when a 30 per cent solution of H_2O_2 was allowed to act on hæmozoin seen in sections, the pigment was disintegrated, while when a 3 per cent solution was used distinct bleaching occurred before disintegration. The bleaching was seen to commence at the periphery and gradually extended into the centre of the granules. This change may be due either to simple bleaching, or to the decomposition of the pigment. Brown concludes that it is the latter change which occurs. Basu (1921) reports, however, that this reagent had no such action on malarial pigment even after 24-48 hours.

Hueck (1921) states that malarial pigment is decolorised by oxidising agents. Glasunow (1925) found that when H_2O_2 was allowed to act on extracted malarial pigment, there was an almost instantaneous decolorisation, with the production of a solution which was quite clear and completely colourless. The decolorising reaction could not be reversed by the action of reducing agents. He concluded, like Brown (1911a), that the action was therefore not a simple bleaching effect, but a decomposition of the pigment.

Seayfarth (1926) records that hæmozoin is bleached and broken up by 30 per cent solution of H_2O_2 , and Wats and White (1932) found that bleaching occurred after prolonged treatment (24 hours or so) with this reagent. The latter workers state that the granules are still visible but colourless, and that the colour cannot be restored by various reducing agents.

It has been shown in our previous work (Ghosh and Sinton, 1934) that H_2O_2 decomposes hæmozoin and hæmatin, setting free non-ionisable iron in an ionisable form. This reaction shows that the effect is not purely a bleaching one, but is due to a decomposition of the pigment.

Brown (1911a) also noted that hæmatin was more quickly decomposed when in a moist state than when dry, and we have been able to confirm this

observation with both hæmatin and hæmozoin. If H_2O_2 be added to a few drops of an aqueous suspension of either hæmatin or hæmozoin, these pigments are decolorised in a short time. When, however, slides coated with dry films of these pigments are dipped in H_2O_2 , the time taken for complete decolorisation is much longer. The interval is longest with hæmozoin enclosed in parasites, due partly to the denaturisation which makes the protein coating less permeable, and partly to the decomposition of the H_2O_2 by this protein before the pigment is reached by the reagent. These factors may be responsible for the failure to obtain decolorisation reported by Basu (1921), or this may have been due to the action of the fixative used on his sections.

As would be expected from the decomposition caused by the oxidising action of H_2O_2 , the colour of the pigment was not restored by reducing agents.

It is concluded, as the result of our experiments, that the decolorising action of H_2O_2 on malarial pigment is due to the decomposition of this substance rather than to a simple bleaching action.

(b) Permanganate of potash.

Brown (1911) reports that malarial pigment is not bleached by $\frac{1}{4}$ per cent solution of permanganate of potash, even after acting for 48 hours. A similar statement is made by Basu (1921).

We tried the effect of a neutral N/10 solution of this reagent on dried parasite hæmozoin, but observed no action even after 48 hours. If, however, the oxidising solution be acidified with H_2SO_4 , and slides coated with a film of the pigment are immersed in it for 48 hours, the pigment is decomposed. This was shown by the fact that such films were cleared up by the action of oxalic acid after withdrawal.

(c) Potassium chlorate.

A neutral saturated solution of potassium chlorate was also found to have no decolorising action on either hæmatin or hæmozoin within the period of observation (6 hours). If, however, the chlorate solution be acidified with HCl, both pigments are completely bleached within 2 hours.

(d) Ammonium persulphate.

Aqueous suspensions of both pigments are slowly decolorised by ammonium persulphate.

A few drops of a suspension of the pigment being tested, were added to 3 c.c. of water and a pinch of ammonium persulphate added three or four times at intervals of about an hour. The suspension was decolorised in 5-6 hours.

(e) Summary.

Hæmozoin has been found to be decolorised by the action of several different oxidising agents, but the colour is not restored by the subsequent action of reducing agents. This result shows that a decomposition of the pigment has taken place.

(ii) REDUCING AGENTS.

In the first paper of this series (Sinton and Ghosh, 1934) it was shown that the sulphides of sodium and ammonium can reduce hæmozoin as well as hæmatin. Ghosh and Sinton (1934) also used ammonium sulphide with success in decomposing these pigments so that their non-ionisable iron was unmasked.

The action of other reducing agents was tried on solutions of these two pigments in caustic potash and in pyridine.

It has been observed by Sinton and Ghosh (1934) that reduced hæmozoin and reduced hæmatin can combine with proteins, with ammonia, and with pyridine to form hæmochromogens, which show two absorption bands in the green. This property has been utilised to follow the action of the various reducing agents investigated.

A small amount of albumen (egg-white) had to be added to the alkaline hæmatin solutions, to provide the protein with which it is necessary for the products of reduction to combine so that the hæmochromogen bands can be demonstrated. This was unnecessary in the case of hæmozoin solutions, because these were prepared directly from the malaria parasites, and thus contained some of the parasite protein.

The results obtained with different reducing agents are recorded in Tables II and III.

TABLE II.

The action of reducing agents on aqueous solutions of hæmozoin and of hæmatin in N/10 KOH.

Reducing agent.	HÆMOZOIN.		HÆMATIN.	
	Solution.	Result.	Solution.	Result.
Pyrogallie acid ..	Pigment solution * 3 c.c. <i>plus</i> a pinch of pyro- gallie acid.	No reduction. No hæmo- chromogen bands appeared in 4-5 mins.; the solution afterwards too dark for observa- tion.	As for hæmo- zoin, <i>plus</i> a drop of egg albumen.	As for hæmozoin.
Hydroquinone ..	Pigment solution * 3 c.c. <i>plus</i> a pinch of hydro- quinone.	No reduction. No hæmo- chromogen bands appeared in 3-4 mins; the solution afterwards too dark for observa- tion.	Ditto	Ditto.
Sodium potassium tartrate.	Pigment solution * 3 c.c. <i>plus</i> two pinches of sodium tartrate	No hæmochromogen bands appeared within one hour.	Ditto	Ditto.
Ferrous sulphate	Pigment solution * 3 c.c. <i>plus</i> two pinches of ferrous sulphate	Hæmochromogen bands appeared within 2-3 mins.	Ditto	Ditto.
Glucose ..	Pigment solution * 3 c.c. <i>plus</i> a pinch of glucose.	No hæmochromogen bands appeared within one hour	Ditto	Ditto.
Egg albumen ..	Strong hæmozoin solution 1 c.c. 50 per cent KOH 1 c.c., 10/N KOH 1 c.c. Shaken and boiled.	On boiling hæmo- chromogen bands appeared within 5-6 mins.	Ditto	Ditto.

* The pigment solution used consisted of 1 c.c. of a strong solution of either hæmozoin or hæmatin in N/10 KOH, *plus* N/10 KOH 2 c.c., so that an excess of alkali was present.

TABLE III.

The action of various reducing agents on haemozoin and on hæmatin dissolved in pyridine.

Reducing agent.	HÆMOZOIN.		HÆMATIN.	
	Solution.*	Result.	Solution.*	Result.
Pyrogallie acid ..	Pigment solution 2 c.c. <i>plus</i> a pinch of pyrogallie acid.	Pyridine hæmochromogen bands appeared in 2-3 mins.	As for hæmozoin.	As for hæmozoin.
Hydroquinone ..	Pigment solution 2 c.c. <i>plus</i> a pinch of hydroquinone.	Ditto	Ditto	Ditto.
Potassium ferrocyanide, in concentrated aqueous solution.	Pigment solution 2 c.c. <i>plus</i> 0.5 c.c. ferrocyanide solution.	Only the α pyridine hæmochromogen band seen in 3-4 mins.	Ditto	Ditto.
Sodium potassium tartrate.	Pigment solution 2 c.c. <i>plus</i> two pinches of tartrate <i>plus</i> 0.5 c.c. water.†	Ditto	Ditto	Ditto.
Ferrous sulphate	Pigment solution 2 c.c. <i>plus</i> two pinches of sulphate <i>plus</i> 0.5 c.c. water.†	Both bands of pyridine hæmochromogen seen within 3 mins.	Ditto	Ditto.
Glucose ..	Pigment solution 2 c.c. <i>plus</i> two pinches of glucose <i>plus</i> 0.5 c.c. of water.†	No pyridine hæmochromogen bands appeared.	Ditto	Ditto.

* The pigment solution consisted of 1 c.c. of a strong solution of either hæmozoin or hæmatin in pyridine *plus* 1 c.c. of pure pyridine.

† The water was added to help the solution of the reducing agents, which were insoluble in pyridine.

From these records, it is seen that in dilute caustic potash solution, it was only with ferrous sulphate that reduction was obtained. Egg albumen in the presence of strong KOH solution can, however, reduce hæmatin rapidly when the solution is boiled. As the hæmozoin solutions used already contained protein, they were also reduced when boiled.

Glasunow (1925) was unable to detect any reduction of hæmozoin with pyrogallie acid in alkaline solution. Our results are in agreement with his observations. In pyridine solution, however, it was found that all the reagents tested, except glucose, can reduce both hæmozoin and hæmatin. Evidently,

therefore, these two pigments are easier to reduce when dissolved in pyridine than when in alkaline solution. It may be noted that each reducing agent tried produced the same effect on both pigments, as would be expected if they were the same compounds.

III. SOME OPTICAL PROPERTIES OF HÆMOZOIN AND HÆMATIN.

(i) SPECTROPHOTOMETRIC MEASUREMENTS.

In our previous work (Sinton and Ghosh, 1934) the spectroscopical appearances presented by hæmozoin in a variety of different solvents were studied. No differences could be detected between these and the appearances shown by hæmatin in similar solutions. These findings gave strong support to the contention that these two pigments are identical. A more detailed quantitative comparison of the capacity of such solutions to absorb light was expected to furnish further evidence on this point. Comparative spectrophotometric measurements were, therefore, undertaken with some of these solutions. So far as we have been able to ascertain, no such measurements have been recorded previously.

(A) Methods and estimations.

The spectrophotometer of Königsberg (1901), as modified by Martins and Grunbaum (1903), was used in this investigation. The following is a brief description of the working of the instrument.*

The instrument.—Parallel rays from the same portion of the source of light illuminate the collimator opening, which is diaphragmed so as to give two slits. The rays, therefore, emerge from the collimator as two parallel pencils of light. These traverse the optical system of the instrument, where they are dispersed, polarised, and brought closer to each other. They finally pass through a narrow ocular slit. On looking through the eyepiece of the telescope used for observing the rays, there are seen two contiguous semi-circular patches of light of the same colour. These correspond to the two parallel rays.

The telescope can be rotated on a vertical plane by a screw provided with a graduated head and moving along a scale. This enables a narrow band of any desired portion of the spectrum to be brought under observation. The position of the telescope can be read from the scale, and each position corresponds to a definite mean wave-length region of the spectrum.

Calibration of the telescope scale.—Before making any observations, it is necessary to calibrate the scale to determine exactly to what region of the spectrum each reading corresponds. This calibration is done by observations of monochromatic radiations of known wave length. Thus in our work the observations shown in Table IV were made.

From these observations a curve was plotted, from which the mean wave length corresponding to any scale reading of our instrument could be determined.

* For a more detailed description the reader is referred to the work of Sheppard (1914).

TABLE IV.

The line spectrum of					Wave length (λ)	Scale reading.
Helium red	6678	4'965
Hydrogen red	6563	4'908
Helium yellow	5876	4'545
Mercury yellow	5790	4'493
Mercury green	5461	4'248
Helium green	5015	3'761
Hydrogen green	4861	3'603

Theory and calculations.—As already mentioned, on looking through the telescope two adjacent semi-circular patches of light are seen, corresponding to the two parallel rays of light. These are polarised at right angles to each other, and one of them can be used as a standard with which the other can be compared. Differences in the intensity of the two patches can be regulated by movements of a Nicol's prism adjustable on a graduated circle.

If I_0 be the intensity of the standard light and I that of the other one, and if a be the angle through which the Nicol's prism has to be rotated to produce an equality of illumination, starting from zero, then these three quantities are related by the expression

$$\frac{I_0}{I} = \tan^2 a \dots \dots \dots (1)$$

Thus if $a = 45^\circ$, then $\tan a = 1$, and under this condition I_0 is equal to I , i.e., the two rays are of equal intensity.

In practice, therefore, it is convenient to adjust the axis of the instrument so that when the Nicol's prism is turned from zero to 45° , the two adjacent semi-circles of light appear of equal intensity, when viewed through the telescope. After this preliminary adjustment, the solution, the absorption coefficient of which it is desired to measure, is poured into a rectangular glass vessel. This is placed in the path of one of the rays so that the angle of incidence is a right angle.

If as before I be the intensity of the transmitted light, I_0 that of the standard, and a the angle through which the Nicol's prism has to be rotated to make the two fields equally bright, then, as in equation (1)

$$\frac{I_0}{I} = \tan^2 a$$

$$\text{or } \log_e \frac{I_0}{I} = 2 \log_e \tan a$$

$$\text{or } 2.303 \log_{10} \frac{I_0}{I} = 2.303 \times 2 \log_{10} \tan a \dots \dots \dots (2)$$

Assuming Beer's law of absorption to hold good, at least for the narrow ranges of concentration used in our experiments, it follows that

$$\frac{I}{I_0} = e^{-kcd} \dots \dots \dots (3)$$

In equation (3) I and I_0 have the same significance as in equation (2), while c is the concentration of the solute, d the thickness of the solution traversed by the light, and k is a constant depending on the nature of the solute and the wave length of light.

It follows from equation (3) that

$$\begin{aligned}\log_e \frac{I}{I_0} &= e^{-kcd} \\ \text{or } \log_e \frac{I_0}{I} &= kcd \\ \text{or } 2.303 \log_{10} \frac{I_0}{I} &= kcd \dots \dots \dots (4)\end{aligned}$$

If equations (2) and (4) be now combined, one gets

$$2.303 \log_{10} \frac{I_0}{I} = kcd \quad 2.303 \sim 2 \log_{10} \tan \alpha \dots \dots (5)$$

and hence $\log_{10} \frac{I_0}{I}$, and therefore kcd , can be calculated from the experimentally determined values of α .

(B) Results of spectrophotometric measurements.

(a) With alkaline aqueous solutions of haematin and haemozoin.

The hæmozoin from* a thick suspension of monkey malaria parasites* was dissolved out with an aqueous $N/2 \text{ Na}_2\text{CO}_3$ solution. Any undissolved parasite remains were removed by centrifugalisation. Pure hæmatin was dissolved in a similar solvent, and the two solutions were adjusted so as to be as nearly as possible of equal strength, so far as could be judged colorimetrically.

A rectangular glass vessel was filled with the hæmozoin solution and placed in the path of one of the pencils of light in the manner already described. The Nicol's prism was rotated until the two fields appeared equally bright, and the angle α was noted. The position of the telescope was also read on the scale.

The telescope was then moved slightly so as to bring a new strip of the spectrum under observation. If the two fields were not of equal intensity, the Nicol's prism was again adjusted and the new angle recorded. This process was repeated so as to obtain a series of values for α corresponding to different positions of the telescope, and so to different mean wave-length regions of the spectrum.

The glass vessel was then carefully washed with distilled water, dried and filled with hæmatin solution. By the same method, a series of values for α were again determined, corresponding to different positions of the telescope. In these experiments the absorption of light from the Na_2CO_3 solution was found to be negligible.

The results of these two series of observations are shown in Table V. From these data a curve for each pigment has been constructed having $\log_{10} \frac{I_0}{I}$ as ordinate and the wave length (λ) of the light in the portion of the spectrum examined as abscissa (*vide* Chart 1).

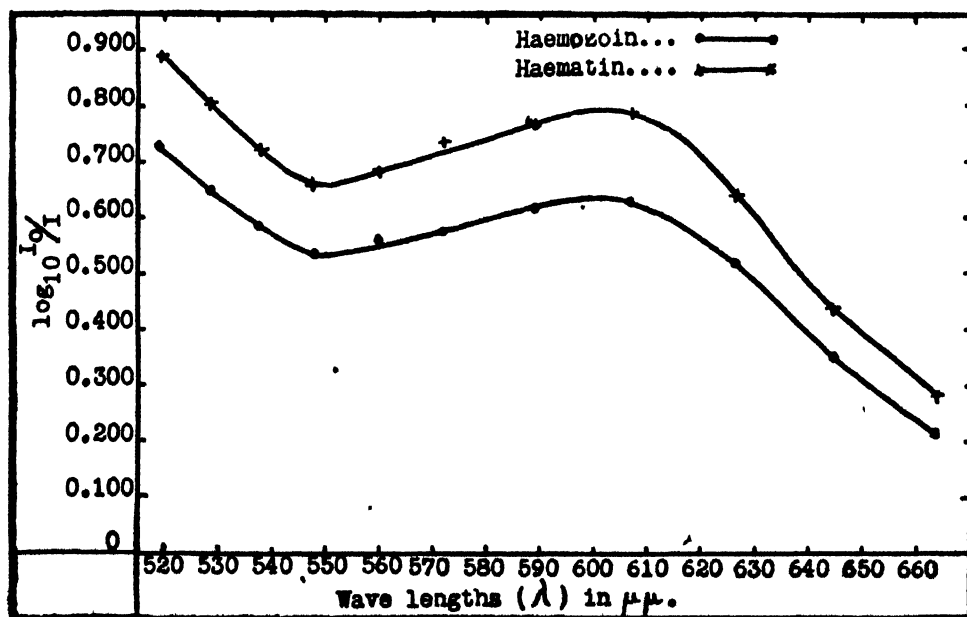
* This suspension was prepared by the technique described in a previous paper (Sinton and Ghosh, 1934).

TABLE V.

Spectrophotometric measurements recorded with solutions of haemozoin and haematin in N/2 Na₂CO₃ (aqueous solution).

Scale reading.	Corresponding wave length (λ) in $\mu\mu$.	HÆMOZOIN.		HÆMATIN.	
		α in degrees.	$2 \log_{10} \tan \alpha$ or $\log_{10} \frac{I_0}{I}$.	α in degrees.	$2 \log_{10} \tan \alpha$ or $\log_{10} \frac{I_0}{I}$.
3.95	519.0	66.5	0.7234	70.3	0.8922
4.05	528.5	64.7	0.6510	68.5	0.8092
4.15	538.0	63.0	0.5858	66.5	0.7234
4.25	548.0	61.6	0.5346	65.0	0.6628
4.35	559.5	62.3	0.5598	65.5	0.6824
4.45	572.0	62.8	0.5782	66.7	0.7318
4.55	589.0	63.8	0.6158	67.5	0.7654
4.65	607.0	64.2	0.6316	68.0	0.7872
4.75	626.5	61.2	0.5196	64.6	0.6468
4.85	645.0	56.5	0.3566	59.0	0.4422
4.95	664.0	52.0	0.2144	54.0	0.2272

CHART 1.



Curves of spectrophotometric measurements recorded with aqueous solutions of hæmozoin and hæmatin in N/2 Na₂CO₃ solution.

If the curves be studied, it will be noted that both show the same characteristic features. If one start from the blue end of the spectrum, as the red end is approached $\log_{10} \frac{I_0}{I}$ at first diminishes, reaches a minimum about $550 \mu\mu$, increases again to reach a maximum about $605 \mu\mu$, and then diminishes again. The maximum about $605 \mu\mu$ corresponds to the absorption band of alkaline hæmatin between $625 \mu\mu$ and $595 \mu\mu$. The increase in the value of $\log_{10} \frac{I_0}{I}$ in the region of wave lengths smaller than $550 \mu\mu$ is due to the general absorption of light by alkaline hæmatin, as noted qualitatively in our previous work (Sinton and Ghosh, 1934). If the $\log_{10} \frac{I_0}{I}$ values of the hæmozoin solution be multiplied by 1.24, then the two curves coincide with each other within the limits of experimental error.

If c_1 and c_2 be the concentrations of the hæmozoin and the hæmatin solutions respectively, and I_1 and I_2 be the intensities of the light transmitted by these solutions for a particular wave length, then for the hæmozoin solution

$$2.303 \log_{10} \frac{I_0}{I_1} = k_1 c_1 d \dots\dots\dots (6)$$

and for the hæmatin solution

$$2.303 \log_{10} \frac{I_0}{I_2} = k_2 c_2 d \dots\dots\dots (7)$$

where k_1 and k_2 are constants characteristic of hæmozoin and of hæmatin respectively, and d is the thickness of the solution, which is maintained the same throughout. Therefore it follows that

$$\frac{\log_{10} \frac{I_0}{I_1}}{\log_{10} \frac{I_0}{I_2}} = \frac{k_1 c_1 d}{k_2 c_2 d} = \frac{k_1 c_1}{k_2 c_2} \dots\dots\dots (8)$$

It has been found experimentally, however, that

$$\frac{\log_{10} \frac{I_0}{I_2}}{\log_{10} \frac{I_0}{I_1}} = 1.24, \text{ therefore } \frac{k_2 c_2}{k_1 c_1} = 1.24.$$

Since the same solutions were used in all the experiments, $\frac{c_2}{c_1}$ must be constant

and consequently $\frac{k_2}{k_1}$ should also be constant throughout the range of wave lengths investigated. But k_2 and k_1 vary as the wave length is altered, and it is highly improbable that this variation should maintain the same constant ratio (1.24) over such a range of wave lengths, unless hæmozoin and hæmatin are closely related to each other. The probability is that $k_2 = k_1$ and that hæmatin and hæmozoin are, therefore, the same compound.

(b) *With alkaline alcoholic solutions of hæmatin and hæmozoin.*

The results recorded in the previous experiment were obtained with a solution of hæmozoin extracted directly from malarial parasites. It was necessary to confirm these results by examining a solution of purified hæmozoin.

An aqueous suspension of pigmented parasites was acidified with HCl and extracted with a mixture of ether and alcohol. The hæmozoin extracted was passed into the ether-alcohol layer and was removed with it. The extraction was repeated several times. The resultant ether-alcohol extract was then concentrated by evaporation, first at room temperature and then at 37°C. in an incubator. The precipitate obtained was washed in distilled water by centrifugalisation, dissolved in N/10 aqueous solution of NaOH, and finally reprecipitated with an excess of oxalic acid. On the following day the oxalic acid was removed from the precipitate by washing with distilled water. The acid dissolved out any ionisable iron present, and this was removed in the washings.

The hæmatin used for comparison was obtained as described by Sinton and Ghosh (1934), and, after dissolving in N/10 NaOH, was treated with oxalic acid in the same manner as was the hæmozoin.

The hæmozoin and the hæmatin were then dissolved separately in a solvent mixture consisting of 50 c.c. absolute alcohol and 50 c.c. of N/10 aqueous solution of NaOH.

The measurements of the absorption of light in the different regions of the spectrum were made as in the previous experiment. The absorption of light by the solvent was found to be negligible. The results of these observations are recorded in Table VI.

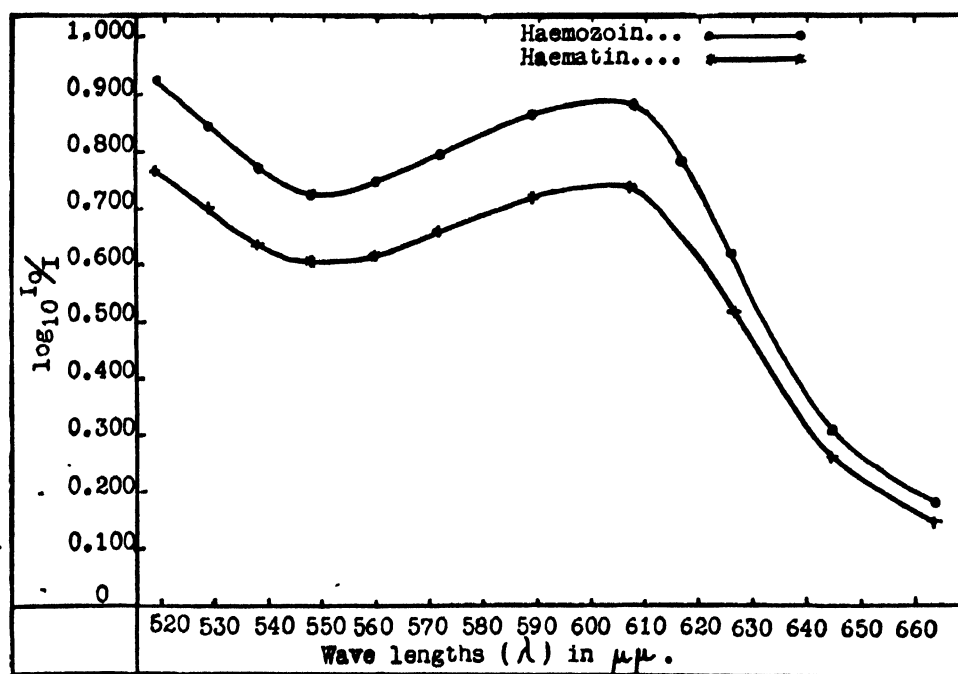
TABLE VI.

Spectrophotometric measurements recorded in solutions of hæmozoin and hæmatin in N/20 NaOH (alcoholic solution).

Scale reading.	Corresponding wave length (λ) in μ .	HÆMOZOIN.		HÆMATIN.	
		α in degrees.	$2 \log_{10} \tan \alpha$ or $\log_{10} \frac{I_0}{I}$.	α in degrees	$2 \log_{10} \tan \alpha$ or $\log_{10} \frac{I_0}{I}$.
3.95	519.0	71.0	0.9260	67.5	0.7654
4.05	528.5	69.4	0.8498	66.0	0.7028
4.15	538.0	67.7	0.7740	64.0	0.6354
4.25	548.0	66.6	0.7276	63.5	0.6042
4.35	559.5	67.1	0.7484	63.7	0.6120
4.45	572.0	68.2	0.7958	65.0	0.6628
4.55	589.0	69.8	0.8686	66.4	0.7192
4.65	607.0	70.5	0.8922	66.9	0.7420
4.70	617.0	68.0	0.7872
4.75	626.5	64.0	0.6236	61.2	0.5200
4.85	645.0	55.2	0.3162	53.5	0.2612
4.95	664.0	51.0	0.1834	50.0	0.1524

As in the previous experiments these results were plotted as curves (*vide* Chart 2), with λ as abscissa and $\log_{10} \frac{I_0}{I}$ as ordinate.

CHART 2.



Curves of spectrophotometric measurements recorded with alcoholic solutions of hæmozoïn and hæmatin in N/20 NaOH Solution.

These curves are of the same type as those obtained with N/2 Na₂CO₃ in aqueous solution, except that here the maximum and minimum are more sharply defined. This agrees with the findings reported in our previous paper (Sinton and Ghosh, 1934) that the addition of alcohol makes the alkaline hæmatin band between 625 μμ and 595 μμ more clearly demarcated.

Both the curves show the same characteristic features. $\log_{10} \frac{I_0}{I}$ is minimum at about 550 μμ and maximum at about 605 μμ. After passing the maximum, $\log_{10} \frac{I_0}{I}$ diminishes rapidly as λ increases. If the $\log_{10} \frac{I_0}{I}$ values of hæmatin are multiplied by 1.20, then the two curves coincide within the limits of experimental error.

As discussed previously in equations (6), (7) and (8), for a given wave length

$$\frac{\log_{10} \frac{I_0}{I_2}}{\log_{10} \frac{I_0}{I_1}} = \frac{k_2 c_2 d}{k_1 c_1 d} = \frac{k_2 c_2}{k_1 c_1} \quad (9)$$

where I_2 , k_2 and c_2 refer to the hæmozoin solution, and I_1 , k_1 and c_1 refer to the hæmatin solution.

It has been found, however, experimentally over the whole range of wave lengths investigated, that

$$\frac{\log_{10} \frac{I_0}{I_2}}{\log_{10} \frac{I_0}{I_1}} = 1.20, \text{ and therefore } \frac{k_2 c_2}{k_1 c_1} \text{ has got the same value (1.20)}$$

throughout the range investigated.

By a colorimetric comparison of the iron contents of the same volume of the two solutions*, $\frac{c_2}{c_1}$ has been determined and found to be 1.22. Therefore $\frac{k_2}{k_1} = 1$ throughout, and hence the two substances are in all probability the same compound.

Summary.

Spectrophotometric measurements have been made of solutions of hæmozoin in aqueous sodium carbonate and in alcoholic caustic potash. The curves of $\log_{10} \frac{I_0}{I}$ against the wave length (λ) have been plotted and these have been found to be of an exactly similar type to those of hæmatin in similar solutions. In the alcoholic potash solutions the absorption constants k_2 and k_1 have been calculated and found to be identical throughout the range of wave lengths observed.

These observations add confirmatory evidence to the conclusion that hæmozoin and hæmatin are the same compound.

(ii) REFRACTILITY OF GRANULES OF HÆMOZOIN AND OF HÆMATIN.

Schaudinn (1902) reported that hæmozoin granules are doubly refractile in polarised light, and that this property enables them to be detected as soon as they appear as scarcely perceptible granules. Langeron (1921) also states that both hæmatin and hæmozoin are doubly refractile.

The occurrence of this property has been disputed by Kaiserling (1910). Glasunow (1925) was also unable to confirm the observation of Schaudinn. He states that the source of error in Schaudinn's work appears to be due to an incomplete lateral darkening, in consequence of which conditions analogous to those of darkground illumination were created.

* In these determinations, 100 c.c. of each of the alkaline solutions were poured into separate porcelain basins, neutralised with HCl and evaporated on a water bath. When nearly dry they were each treated with 3 c.c. of H_2O_2 solution containing a few drops of HCl, and again slowly evaporated to dryness. The latter treatment with acidified H_2O_2 was repeated once more and the mixture evaporated completely to dryness. The residue was extracted with 1.5 c.c. of HCl and 3 c.c. of water, and finally the volume of each solution was made up to 15 c.c.

The two solutions were then centrifuged and 9 c.c. of the clear supernatant fluid were withdrawn from each. These portions were placed in separate vessels and 1 c.c. of concentrated KCNS was added to each. The depth of colour was compared in a colorimeter and the ratio of c_2 to c_1 was found to be 1.22.

Seyfarth (1926), however, reports that with polarised light the pigment of parasites in fresh blood is doubly refractile, and shows a beautiful display of colours against the background of a uniformly dark parasite. He considers that Schaudinn's observations have been questioned wrongly, because he (Seyfarth) found that the pigment loses this double refractility in fixed and stained preparations.

We have examined suspensions of malarial parasites under the polarising microscope, and have been unable to determine any signs of double refractility in the pigment. A similar result was obtained with hæmatin. On the other hand, hæmin crystals showed this property.

(iii) DICHOISM.

Gardner and Buckmaster (1913) and Robertson (1920) note that alkaline solutions of hæmatin are dichroic, as shown by the green coloration of thin layers and the red appearance of thick ones.

Our observations have also shown that alkaline solutions of both hæmozoin and of hæmatin display this dichroic character.

IV. CRYSTALLISATION OF HÆMOZOIN AND OF HÆMATIN.

(i) TEICHMANN'S CRYSTALS.

If hæmozoin and hæmatin be identical, it should be possible to prepare hæmin crystals from hæmozoin, as can be done from hæmatin.

Glasunow (1925) reports that he succeeded in preparing crystals of hæmatin chloride (hæmin) and hæmatin iodide from pigment extracted with 0.04 per cent alcoholic potash solution from malarial splenic tissue fixed in Orth's fluid.

It has been noted in the earlier parts of this work that experiments conducted with pigment extracted from fixed tissues may give fallacious or inconclusive results. To test Glasunow's results some normal monkey blood was fixed in Orth's fixative and, after washing thoroughly with distilled water, was extracted with 0.04 per cent alcoholic potash. On spectroscopical examination the extract showed faint absorption bands of alkaline hæmatin. The results reported by Glasunow cannot, therefore, be considered to be conclusive.

A suspension of highly pigmented malarial parasites was repeatedly washed and extracted over long periods with distilled water, until no trace of hæmoglobin was detectable either naked eye or spectroscopically in the washings.

An acid solution was prepared consisting of hydrochloric acid 0.2 c.c., water 0.8 c.c., glacial acetic acid 1.0 c.c. and absolute alcohol 1.0 c.c.

A little of the pigment was taken on a glass slide, covered with a drop or two of the acid solution, and heated over a flame. As the acid solution evaporated more was added, and finally it was boiled for 30–40 seconds. After cooling, the slide was examined under the microscope and was found to show numerous typical crystals of hæmatin chloride (hæmin).

Hæmin iodide crystals were prepared in a similar manner, except that in the acid solution 0.2 c.c. of hydrochloric acid was replaced by 0.3 c.c. of

hydroiodic acid. As reported by Glasunow (1925) the iodide crystals were obtained more easily than the chloride ones.

In both experiments the crystals observed were indistinguishable from those obtained from freshly prepared acid hæmatin by the same technique.

(ii) HÆMOCHROMOGEN CRYSTALS.

When a mixture of hæmoglobin with pyridine is treated with a suitable reducing agent, such as ammonium sulphide, needle-like ruby-red crystals of pyridine-hæmochromogen are formed. An attempt was therefore made to prepare such crystals from hæmozoin.

A concentrated solution of hæmozoin in pyridine was prepared. Two drops of this were placed on a glass slide and further concentrated by evaporation over a small flame. A drop of ammonium sulphide was then added, the mixture covered immediately and examined under the microscope. Numerous small ruby-red needle-like crystals could be detected in such preparations.

(iii) SUMMARY.

Crystals of hæmatin chloride, hæmatin iodide and of pyridine-hæmochromogen have been prepared from hæmozoin. These results add further evidence as to the identity of hæmozoin with hæmatin.

V. SUMMARY AND CONCLUSIONS.

Under the conditions of our experiments it was found

- (a) that the rate of solution of purified hæmozoin was similar to that of hæmatin;
- (b) that the decolorising action of certain oxidising agents on hæmozoin is not a true bleaching action but caused by a decomposition of the pigment;
- (c) that the reducing agents tried produced the same effect on both the pigments. It is easier to reduce the pigments when dissolved in pyridine than when in alkaline solution;
- (d) that spectrophotometric measurements of solutions of hæmozoin and of hæmatin suggest strongly that these two substances are identical;
- (e) that neither hæmozoin nor hæmatin showed double refractility under polarised light, though hæmin crystals did show this property;
- (f) that alkaline solutions of these two pigments were dichroic;
- (g) that crystals of hæmatin chloride and hæmatin iodide can be formed from hæmozoin and these crystals are indistinguishable from those obtained from hæmatin by a similar technique; and
- (h) that it is possible to form pyridine-hæmochromogen crystals from hæmatin, as also from hæmozoin.

As a result of these and our previous experiments, we consider that the pigment found in *P. knowlesi*, a malarial parasite of lower monkeys, is indistinguishable from hæmatin.

We wish to express our thanks to Professor M. R. Chatterjee, Professor of Geology, Presidency College, Calcutta, for allowing one of us to undertake the polarising observations in his laboratory, and to Professor J. C. Ghosh,

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STUDIES IN UNTREATED MALARIA.
NUMERICAL STUDIES OF THE PARASITES IN RELATION TO THE
FEVER.

BY

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[28th March, 1934]

IN a previous paper (Lowe, 1934), we have discussed at length the clinical and the blood findings made in a series of untreated cases of malaria due to fresh infections by *P. vivax* and *P. falciparum*.

The following is a discussion of the numerical studies carried out in the same series of untreated cases. The data for this study are :—

1. Total parasite counts made by Sinton's (1924) method at 8 a.m., 12 noon, 4 p.m., and 8 p.m., and sometimes more frequently during the period of the fever.

2. Differential parasite counts made at the same time as the total parasite counts, the parasites being classified as rings, trophozoites, schizonts, and gametocytes.

3. A four-hourly temperature chart of each patient.

A. THE NUMBER OF PARASITES IN THE PERIPHERAL BLOOD.

The parasite counts recorded in the present series vary from 20 to 202,000 per cubic millimetre. The highest count which we have found in the literature is 2,800,600 reported by Chopra, Das Gupta and Sen (1932) in a fatal case of malaria due to *P. falciparum*. The parasite counts in malaria vary tremendously with differing circumstances. The principal factors which affect the count are, we believe, as follow :

(1) VARIATIONS IN THE PLASMODIA CAUSING THE FEVER

(a) *The species of Plasmodium causing the malaria.*—In febrile cases 142 counts were made in malaria due to *P. vivax* with an average count of 11,100, and 68 counts were made in malaria due to *P. falciparum* with an average count of 40,800. This difference is striking especially when it is considered that in several cases of malaria due to *P. falciparum*, the parasites practically disappeared from the peripheral blood at certain periods of the fever. Ross and Thomson (1910) gave particulars of 12 counts of *P. vivax* and 35 counts of *P. falciparum*. We have calculated the average count in Ross and Thomson's cases as *P. vivax* 464, and *P. falciparum* 25,770. No clearer proof could be given of the well-recognised fact that during pyrexia in malaria due to *P. falciparum* the average count in the peripheral blood is much higher than in malaria due to *P. vivax*.

(b) *The strain of Plasmodium causing the fever.*—Rudolf (1924), Pijper and Russel (1925), Bunker and Kirby (1925) and Lilly (1925) have all found that different strains of the same parasite vary markedly in virulence. It is very noticeable that in the present series the first cases seen after the onset of the malarial season showed heavier infection than those seen later on. As time went on, infection of *P. vivax* and *P. falciparum* both became milder, suggesting that the strains of both parasites became less virulent.

(2) VARYING CONDITIONS IN THE PATIENT.

James (1926) and other workers have shown that different patients vary in their susceptibility and resistance to malarial infections. We have found the same thing in the present series of cases and consider that the following factors are of importance.

(a) *The age of the patient.*—It is well recognised that malaria is a much more serious condition in children than in adults. Our general experience is that children show heavier infection in the peripheral blood than do adults. Only two children are included in the present series. One showed a count of 105,000 *P. vivax*, a very high count for this parasite, and the other showed a count of 130,000 *P. falciparum*.

(b) *Previous malarial infection.*—This in endemic countries is associated with the age of the patient, as few people can live long in such countries without being infected. Previous infections apparently tend to make subsequent fresh infections less severe.

(c) *The nature of the infection* (whether fresh infection or relapse).—Fresh infections are associated with higher parasite counts than are relapses. In the present series are included 37 fresh infections by *P. vivax* with an average count of 11,290 after the first rigor, and 3 relapsing infections with an average count after the first rigor of 2,247. The only relapsing infection with *P. falciparum* showed a count of 4,900 in marked contrast to the high counts found in fresh febrile infections which averaged 40,080.

(d) *Intercurrent diseases.*—In the present series in six cases there was an intercurrent infection of influenza. In these cases the average parasite count after the first rigor was for *P. vivax* 3,360 and for *P. falciparum* 8,400, in contrast to an average in uncomplicated cases of 11,200 and 40,080 respectively. It seems probable that in these cases there was a mild infection of malaria

which might never have shown itself clinically if it had not been aggravated by influenza. It is worthy of note that these influenza cases showed fever out of all proportion to the parasite count.

(3) THE TIME OF EXAMINATION.

In malaria due to *P. vivax*, parasite counts at the first rigor and for the first two or three days tend to be high, and then diminish gradually. Also counts taken just after a rigor are higher than those taken some time after the rigor. In the later stages of the infection, rigors may be followed by a diminution in the number of parasites in the peripheral blood.

In malaria due to fresh infection of *P. falciparum*, the number of parasites found in the peripheral blood varies tremendously not only from day to day but from hour to hour, and this variation is much more marked than in malaria due to *P. vivax*. This phenomenon is well illustrated in Table I.

TABLE I.
Malaria due to P. falciparum.

Patient E Date.	Time.	Temperature, °F.	Parasite count per c.mm.
Aug. 17	4 p.m.	104°4	66,000
	8 p.m.	101°0	50,000
Aug 18	8 a.m.	99°0	48,000
	12 noon	99°0	10,000
	4 p.m.	100°0	9,000
	8 p.m.	101°0	360
Aug 19	8 a.m.	102°0	1,900
	12 noon	106°0	130,000
	4 p.m.	106°0	92,400

(Quinine given.)

In malaria due to either *P. vivax* or *P. falciparum*, the time of examination influences markedly the number of parasites found.

All the factors mentioned must be considered when discussing the significance of the number of parasites found in the peripheral blood.

B. THE PARASITES IN THE PERIPHERAL BLOOD AND THE FEVER.

In discussing and basing conclusions on the findings in the peripheral blood in malaria, there is one great fallacy. As Manson said 'The forms seen in the peripheral are but a part of the great drama that is being enacted in the spleen and in other internal organs'. The peripheral blood findings may sometimes be a guide as to what is happening in the body as a whole, but sometimes they most certainly are not. It is well known that in malignant tertian malaria in its severe progressive forms, parasites may diminish and

even disappear from the peripheral blood while they are tremendously numerous in the internal organs. Also mature forms of *P. falciparum* are rarely found in the peripheral blood, only early trophozoites usually being seen. There is some evidence that a tendency towards a similar phenomenon may be found in other forms of malaria. Interpretations of the meaning of findings in the peripheral blood need therefore be made with a caution which is not always exercised by writers on malaria. Keeping this possible fallacy in mind we proceed to discuss the relation between the parasites in the peripheral blood and the fever.

Under this heading three different questions arise :

1. Is the fever related to a particular phase in the life cycle of the parasite ?
2. How many parasites are necessary to cause fever ?
3. Is the degree of fever produced proportional to the number of parasites ?

The first question may be said to have been definitely answered. All workers are agreed that fever is due to the liberation of merozoites into the blood by the bursting of mature schizonts, and all our present findings support this view.

The second and third questions may be regarded as still open. Regarding the number of parasites and the occurrence of fever Ross and Thomson (1910) as the result of their investigation stated 'There would seem to be a very decided correlation between the number of asexual plasmodia found in the peripheral blood and fever'. This statement was confirmed by Rudolf and Ramsay (1927) who found this same relationship in five out of ten cases of artificially-induced malaria due to *P. vivax*. Sinton *et al.* (1931) stated regarding chronic malaria due to *P. vivax* 'There is a distinct relationship between the number of parasites and the occurrence of fever'. Other workers, *i.e.*, Bohm (1918) and Pijper and Russel (1925), have, however, failed to confirm this finding.

The term 'pyrogenic threshold' has been used to indicate the minimum number of parasites per c.mm. which is necessary to cause fever in a particular case. Various workers have tried to establish a more or less constant figure for the pyrogenic threshold for the various species of malarial Plasmodia. We find that while the conception of pyrogenic threshold is valuable in understanding malaria and especially relapse, yet we should not expect its value to be constant in different patients under different conditions. The pyrogenic threshold is markedly affected by most of the factors enumerated above as affecting the number of parasites found in the peripheral blood, and consequently its value varies tremendously.

Sinton *et al.* (1931) summarised the writings of various workers on the pyrogenic threshold and quoted the following :

Naturally-acquired malaria.

Sims (1902)	400 parasites per c.mm.
Ross (1911)	at least 50 parasites per c.mm.
Ross and Thomson (1910)	200 to 500 parasites per c.mm.

Artificially-induced malaria.

- Kortweg (1924) .. Primary infections 1 to 20 parasites per c.mm.
 Secondary infections 200 to 500 parasites per c.mm.
- Grant and Silverston (1926). Fever often starts before the parasites can be found.

In their own investigation in chronic relapsing malaria due to *P. vivax*, Sinton *et al.* find that there is 'a very definite threshold equivalent to about 5,000 young parasites per c.mm. of blood'. They think that the constancy of their findings, which is in marked contrast to the inconstancy of the findings of other workers, may be explained by the nature of their cases, namely British soldiers who have relapsed after treatment and in whom the resistance is probably more or less uniformly low.

In the present investigation we have available three indications of the value of the pyrogenic threshold. Firstly, we can find out the number of young trophozoites present after the first rigor; the resultant figure should be higher than that of the pyrogenic threshold. Secondly, we can find out the number of young trophozoites present after the last rigor in cases undergoing spontaneous arrest; this also should give us a figure higher than that of the pyrogenic threshold. Thirdly, we can record the counts of young trophozoites made after a completed schizogony at an afebrile period; this should give us a figure lower than that of the pyrogenic threshold. From these three figures we should be able to make a rough calculation of the true pyrogenic threshold.

(a) *P. VIVAX.*

The data concerning *P. vivax* are produced in Table II.

Since many of our patients showed two crops of parasites, and only one of these crops could be responsible for the fever, we have not taken the figures of the total parasite count, but from a knowledge of the total and differential parasite counts, we have calculated the number of young trophozoites after the rigor and have taken that figure as an indication of the pyrogenic threshold. It will be seen that the number of young parasites after the first rigor varies markedly from 624 to 101,000 and the average is 11,200. This very high figure is largely caused by a few abnormally high counts. If we ignore these and examine the rest we find that of 22 cases, 17 showed after the first rigor a number of young parasites varying from 600 to 9,000 with an average of 4,656. This figure while giving a rough guide to the pyrogenic index is probably too high and not an accurate guide. These counts we made at the beginning of fever when the multiplication of parasites may be very rapid. To take an example it is possible for the parasites before the first rigor to number 500 and after the first rigor 4,000. It would be very fallacious to say that the threshold value in such a case is 4,000. It may be only 1,000. Also we cannot be sure that there had not been slight fever for a little time before the first examination.

The same calculation in relapsed cases gives figures varying from 1,300 to 3,600 with an average of 2,247.

Calculations based on the last rigor in cases undergoing spontaneous arrest give figures varying from 88 to 4,600 with an average of 1,674 for fresh infections and 366 for relapsed. These figures are also probably too high to indicate the pyrogenic threshold. The number of parasites in every case was much

TABLE II.

*P. vivax.*A. COUNTS AFTER FIRST RIGOR.
FRESH CASES.

Patient.	Total parasite count per c.mm.	Young trophozoites, per cent.	Young trophozoites, number per c.mm.
G. B. ..	12,000	24	2,880
P. ..	10,500	44	4,620
Band ..	4,200	60	2,520
B. P. ..	3,960	60	2,376
R. P. ..	3,500	84	2,940
A. L. ..	8,800	60	5,280
A. G. ..	25,200	50	12,600
M. ..	110,000	92	101,200
M. S. ..	15,000	82	12,300
S. R. ..	1,200	52	624
R. R. ..	38,900	22	8,558
M. P. ..	51,400	49	25,186
N. K. ..	7,920	91	7,207
D. ..	8,000	96	7,680
K. G. ..	6,000	96	5,760
Gn. ..	8,400	74	6,216
A. R. ..	13,680	97	13,270
G. P. ..	18,360	26	4,674
T. P. ..	31,560	20	6,312
G. J. ..	15,600	44	6,864
K. B. ..	3,840	84	3,226
P. Y. ..	12,200	35	4,270

Average 11,200 *

RELAPSED CASES.

G. G. ..	1,380	94	1,317
P. ..	4,800	75	3,600
P. ..	1,920	95	1,824

Average 2,247

B. COUNTS AT LAST FEBRILE PERIOD BEFORE
SPONTANEOUS ARREST.
FRESH CASES.

Patient.	Total parasite count per c.mm.	Young trophozoites, per cent.	Young trophozoites, number per c.mm.
P. ..	9,200	50	4,600
C. N. ..	9,960	20	1,992
B. P. ..	3,960	60	2,376
G. J. ..	1,680	45	656
K. B. ..	1,380	24	331
P. Y. ..	420	21	88

Average 1,674 *

RELAPSED CASES.

G. G. ..	1,200	51	612
P. ..	280	43	120

Average 366 *

C. COUNTS IN AFEBRILE PERIODS.

FRESH CASES.

A. L. ..	1,200	8	96
C. N. ..	240	50	120
B. P. ..	180	50	90
G. J. ..	360	40	144

Average 112 *

RELAPSED CASES.

G. G. ..	180	66	119
Y. Y. ..	50	96	48

Average 84 *

D. SCHIZONT COUNTS IN AFEBRILE PERIODS.

FRESH CASES.

Patient.	Total parasite count per c.mm.	Schizonts per cent.	Schizonts, number per c.mm.
G. J. ..	150	16	24
K. B. ..	60	40	24
P. Y. ..	100	60	60

Average number of schizonts per c.mm. 36.

* These averages refer to number of young trophozoites per c.mm.

reduced before the next schizogony was due, so we cannot be sure that this number, if they had persisted, would not have caused fever. Counts in afebrile periods give figures varying from 24 to 150 with an average of 80.

These findings indicate that the value of the pyrogenic threshold of *P. vivax* varies markedly under different conditions. There are however certain broad indications. Counts below 200 are rarely associated with fever. With counts between 200 and 500 there may or may not be slight fever. We have not seen an afebrile case with 500 or more young trophozoites in the peripheral blood. We would therefore suggest 500 as the rough value of the pyrogenic threshold in most of our cases, though there are some who have shown fever with fewer parasites than this.

This figure 500 is very much lower than that established in chronic malaria by Sinton *et al.*, but there is a marked difference in the type of cases dealt with. Our cases were nearly all fresh infections in Indians of rather poor physique while theirs were chronic relapsing cases in British soldiers. It is worthy of note that in the small number of relapsing cases included in the present series, the pyrogenic threshold seemed to be lower and not higher than in the fresh infections.

(b) *P. FALCIPARUM.*

In the present investigation the information regarding the value of the pyrogenic threshold of *P. falciparum* is limited. We have fourteen counts made after the first rigor in fresh cases, and one count in a relapsed case.

TABLE III.

P. falciparum.

COUNTS AFTER THE FIRST RIGOR
FRESH INFECTIONS.

Patient.	Young trophozoites after first rigor	Patient.	Young trophozoites after first rigor.
E.	66,000	S. R.	8,000
I. B.	76,000	V. L.	27,000
M. E.	18,800	B. D.	22,860
B.	6,400	M.	102,000
R. Y.	9,300	R. J.	48,000
Y. R.	22,000	G. B.	176,000
M. K.	136,000	M. B.	80,000

Average young trophozoites per c.mm. 57,200

RELAPSING INFECTIONS.

G. P. 4,900

It is obvious that most of these figures are much too high to indicate the pyrogenic threshold value. *P. falciparum* produces 32 merozoites as a rule, and thus the counts after the first rigor may be many times greater than the pyrogenic threshold. The lowest counts recorded are 4,900 and 8,000. It would seem that the figure for the pyrogenic threshold of *P. falciparum* is higher than that for *P. vivax*, and the results here quoted suggest that it is below 5,000. Ross and Thomson (1910) express the opinion that 600 to 1,500 may perhaps be adopted as the usual limit. These figures seem rather low but in the absence of more precise information they may be accepted.

C. THE NUMBER OF PARASITES AND THE DEGREE OF FEVER.

Regarding the relationship between the number of parasites and the degree of fever, Ross and Thomson (1910) say :

'It is probable although by no means certain that the resistance to the toxins of plasmodia varies not only in different persons but in the same person at different stages in the course of his infection and under different physiological conditions'.

Sinton *et al.* (1931) quote this with approval and state :

'The recent extensive work with artificially-induced malaria amply confirms this hypothesis. One would not expect that the same number of parasites would cause the same degree of pyrexia in all individuals, but that there might be a distinct relation between these (the number of parasites and degree of fever) in the same individual under the same conditions'.

Table IV gives the results of blood examination before a rigor, the temperature after the rigor, and the count after the rigor in some typical cases of malaria due to *P. vivax*. These results show that in infections with *P. vivax* the relation between the number of parasites and the degree of fever in different patients is not marked. Those patients with the highest counts sometimes tend to show the greatest degree of fever but there are many exceptions to this general tendency. Nevertheless in any one patient an increase or decrease in the parasite count is usually accompanied by an increase or decrease in the fever, and disappearance of the parasites from the peripheral blood is always accompanied by a cessation of the fever.

Table V indicates that in malaria due to *P. falciparum* the relation between the number of parasites *before* the rigor and the degree of fever is little, but there is in most cases a fairly marked relation between the number of parasites immediately *after* the rigor and the degree of fever. This is as one would expect since the mature pre-schizogony forms are all in the internal organs and not in the peripheral blood, and the count before the rigor is no indication of the severity of the infection but the count after the rigor is.

TABLE IV.

Malaria due to P. vivax.

CORRELATION BETWEEN THE NUMBER OF PARASITES AND THE DEGREE OF FEVER.
FRESH INFECTIONS.

Case.	Number of schizonts before rigor.	Temperature, °F., at rigor.	Number of young trophozoites after rigor.
T. N. ..	Not taken	104	19,680
	2,080	105.4	15,540
	240	104	274
P. ..	Not taken	104.6	4,620
	600	103	1,500
	4,128	105.4	4,891
	595	104.6	4,600

TABLE IV—*concl'd.*

Case.	Number of schizonts before rigor.	Temperature, °F., at rigor.	Number of young trophozoites after rigor.
R. P. ..	1,953	104	7,930
A. L. ..	Not taken	101·6	5,280
G. B. ..	6,120	104·6	19,440
	2,464	104	8,190
	7,980	104	3,200
	3,542	104·5	11,656
	1,660	104	14,697
	8,113	104	4,935
M. P. ..	Not taken	102·6	25,186
B. ..	Not taken	103·6	2,520
	380	102·4	2,370
	4,582	105	11,651
	5,439	105	2,990
	13,133	105	436

RELAPSING INFECTIONS.

G. G. ..	Not taken	103	1,297
	160	104·5	1,100
	320	102	336

TABLE V.

Malaria due to P. falciparum.

CORRELATION BETWEEN THE NUMBER OF PARASITES AND THE DEGREE OF FEVER.

FRESH INFECTIONS.

Patient.	Count before rigor.	Temperature, °F., at rigor.	Count after rigor.
E. ..	Not taken	104·4	66,000
	1,900	106	130,000
I. B. ..	Not taken	104·6	76,000
	100	104·8	100
V. L. ..	Not taken	105	27,000
	23,000	104	27,000
S. R. ..	Not taken	101	8,400
	60,000	104	108,000
R. Y. ..	Not taken	103	9,300
	100	105·6	51,000

TABLE V—concl'd.

Patient.	Count before rigor.	Temperature, °F., at rigor.	Count after rigor.
B ..	Not taken 4,800	102°4 104°4	6,400 90,000
M. K. ..	Not taken 74,300	104°5 105	136,000 195,000
R ..	Not taken	103	48,000
Y. R. ..	Not taken 1,440	102 ..	22,800 116,400
S. A. ..	Not taken	105	5,160
M. E. ..	Not taken 2,360	103 105°5	19,800 74,880
M. Y. ..	Not taken	106	36,000
Mub. ..	Not taken	104	102,000
B. D. ..	Not taken	103	22,860
Gor. ..	Not taken	105	176,400
M. B. ..	Not taken	105	80,000
RELAPSING INFECTION.			
G. P.	Not taken	100	4,900

D. VARIATIONS IN THE TOTAL PARASITE COUNT AND THEIR SIGNIFICANCE.

Variations in the parasite counts in the peripheral blood of any patient may be due to one or more of the following general factors :

- (a) Multiplication of the parasites by schizogony.
- (b) Liberation of parasites from the internal organs.
- (c) Destruction of parasites in the peripheral blood.
- (d) Disappearance of parasites into the internal organs.

In discussing variations in the peripheral blood count all these factors must be considered, and, before attributing increases or decreases to multiplication or destruction of parasites, we should try to exclude the possibility of liberation from, or disappearance into, the internal organs.

What evidence have we that the peripheral blood findings are any guide to what is happening in the body as a whole ? The only evidence we have is

that obtained by correlating the general clinical findings with the blood findings over a considerable period in the same patient. When we try to do this in the fresh infections by *P. vivax* and *P. falciparum* here recorded, we immediately find that there is a tremendous difference between the two infections. We find in infections due to *P. vivax* that, when the parasites in the peripheral blood increase in number, the fever tends to rise higher and other symptoms tend to increase. When the parasites decrease, the fever and other symptoms tend to get milder, and when the parasites disappear the fever disappears. We may conclude therefore that the findings in the peripheral blood are a rough guide to what is happening in the body as a whole. Parasites may be liberated from or disappear into the internal organs to a certain extent and thus minor variations in the parasite count may be caused, but this is a minor matter. Marked increase or decrease in the number of parasites in the peripheral blood must mean multiplication or destruction of parasites.

In fresh infections by *P. falciparum* it is a very different story. A tremendous diminution in the parasites in the peripheral blood in the intervals between the rigors is commonly seen, but this is often followed not by a decrease but by an increase in symptoms at the next rigor. The findings in the peripheral blood on any one occasion are often at variance with the clinical findings at the moment of examination. For example at 4 p.m. on August 19th, case B. showed a temperature of 104.8 with less than 100 parasites per c.mm. of blood. At 12 noon on October 4th, case M. K. showed no fever with 75,000 parasites per c.mm. These two cases quoted are certainly exceptional, but experience has shown us that malaria due to *P. falciparum* is a most treacherous disease. Repeated blood examinations often do not reveal what is going on. The battle between the parasites and the defence mechanism takes place in the internal organs, and the peripheral blood findings may give little information regarding the progress of the battle. We have come to regard a sudden marked diminution in the parasites in the peripheral blood as a bad sign, and promptly institute quinine treatment.

Thus we find that variations in the parasite counts of *P. vivax* and *P. falciparum* have different meanings and we shall discuss them separately.

(1) THE EFFECT OF SCHIZOGONY ON THE PARASITE COUNT.

(a) *P. vivax*.

Bohm (1918) reported a case of benign tertian malaria showing 11,000 parasites before the rigor and 41,000 after the rigor, an increase of 3.7 times. Sinton *et al.* (1931) report increases of 4.4, 3.8, and 2.0 times. We believe that one schizont of *P. vivax* may produce twenty or more merozoites. Theoretically therefore it is possible for the parasite count to increase twenty times as the result of schizogony. In practice we find that this does not occur. Table IV is based upon the blood examinations recorded in the present series of cases. As many of our patients show more than one crop of parasites, we cannot take the counts before and after the rigor as indicating the multiplication rate. We have to separate the crops, and take the number of schizonts before the rigor and the number of young trophozoites after the rigor.

TABLE VI.

*The effect of schizogony on the parasite count.**P. vivax.*

Case.	Day of fever.	Number of schizonts before rigor.	Number of young trophozoites after rigor.	Multiplication factor.	Percentage of merozoites destroyed.
T. N. ..	3	2,100	15,500	7.5	62.5
	4	240	274	1.1	94.5
P. ..	3	600	1,500	2.5	87.5
	4	4,100	4,900	1.2	94.0
	5	600	4,600	7.5	62.5
R. P. ..	3	1,960	7,950	4.0	80.0
G. B. ..	2	6,100	19,450	3.2	84.0
	3	2,460	8,190	3.3	83.5
	4	7,980	3,200	0.4	98.0
	5	3,542	11,660	3.0	85.0
	6	1,660	11,700	9.0	55.0
	7	8,110	4,930	0.6	97.0
B. ..	2	380	2,370	6.3	68.5
	3	4,582	11,651	2.5	87.5
	4	5,439	2,990	0.55	97.25
	5	13,133	436	0.03	99.85
G. J. ..	3	1,536	1,720	1.1	94.5
	4	720	736	1.0	95.0
	5	280	44	0.21	99.0
	6	12	nil	nil	100.0
K. B. ..	3	1,224	216	0.18	99.1
	5	30	nil	nil	100.0
P. Y. ..	3	96	88	0.9	95.5
	4	317	60	0.2	99.0
A. G. ..	3	5,070	17,600	3.5	82.5
G. G. ..	3	160	1,100	7.0	65.0
	5	320	336	1.0	95.0
	7	30	nil	nil	100.0
A. L. ..	2	275	96	0.3	98.5

Table VI shows that the results of schizogony are tremendously variable. Of 29 cases after schizogony, an increase in the parasite count was found in 15, a decrease in 12, and no difference in 2. The increases were mostly seen in the early days of the fever and the decreases at a later stage, but there are exceptions to this. The maximum increase observed was a ninefold one and this occurred on the 6th day of the fever. Some cases showed a decrease in the multiplication rate as fever progressed (e.g., patient G. J.). Even under the most favourable conditions 55 per cent of merozoites failed to survive, and

in some cases (G. J., G. G., etc.), at the end of the fever, 100 per cent of the merozoites failed to survive. These findings show that of merozoites produced, most are destroyed before they infect red cells. The destruction rate varies between 50 per cent and 100 per cent according to the circumstances. As fever continues and immunity develops, the destruction rate tends to rise and there may be fewer young trophozoites than there were schizonts. Finally all merozoites may be destroyed.

(b) *P. falciparum* (Table V).

The usual finding is that before a rigor the parasites in the peripheral blood are comparatively few and those that are present are early trophozoites. This shows that schizogony of *P. falciparum* is more or less continuous process, occurring to a mild degree during the whole course of fever. In most cases however there are every other day, or in some cases every day, periods of very heavy schizogony associated with hyperpyrexia and other symptoms.

These periods are practically always associated with a marked increase in the count in the peripheral blood. Case E. shows a count of 1,900 before the fever and 130,000 after. Case R. Y. shows a count of 100 before the fever and 50,000 after. Schizonts are rarely found in the peripheral blood so this increase is obviously due to liberation of young forms by the mature schizonts in the internal organs. It is therefore difficult to estimate the effect of schizogony on the total parasite count. We know that in infections due to *P. vivax*, enormous numbers of merozoites are destroyed immediately after schizogony occurs. Probably the same thing happens in infections by *P. falciparum*. Since *P. falciparum* produces on an average 32 merozoites the mortality rate of free merozoites is probably as high as that of merozoites of *P. vivax*. Even if 96 per cent of merozoites fail to survive the total number of parasites in the body after the rigor will be slightly greater than it was before.

(2) THE PARASITE COUNT IN THE INTERVALS BETWEEN THE RIGORS.

(a) *P. vivax*.

It has long been recognised in a general way that parasites are more numerous in the blood at, and shortly after, the rigor than they are in the intervals between the rigors, but no careful investigation of this phenomenon by numerical methods has been made except by Rudolf and Ramsay (1927). Bohm (1918) recorded a case of benign tertian malaria which showed 87,500 parasites per c.mm. during the fever, 11,000 during the afebrile period between this rigor and the next, and 41,000 at the next febrile period. Sinton *et al.* (1931) state :

‘The fact that in our results the counts made in the inter-pyrexial intervals are mostly lower than those made at the time of pyrexia suggests that a considerable destruction of parasites is taking place at the latter time’.

Perhaps the most striking finding in the cases here recorded is that in every case in the afebrile interval between every two rigors there is a steady and marked diminution in the total parasite count. This is shown clearly in

Table VII to be due to the fact that many of the trophozoites never attain maturity but are apparently destroyed.

TABLE VII.
Percentage of trophozoites attaining maturity.
P. vivax.

Case.	Number of young trophozoites after rigor.	Schizonts present, 40 hours later.	Percentage of young trophozoites reaching maturity.	Notes
T. N. ..	19,500	240	1·2	
P. ..	4,600	4,100	90·0	
	1,500	600	40·0	
	4,900	nil	0·0	
	4,600	nil	0·0	Spontaneous arrest
G. B. ..	19,450	7,980	40·0	
	8,190	3,542	40·0	
	3,200	1,660	50·0	
	11,660	8,117	70·0	
G. J. ..	6,864	720	11·0	
	1,720	208	12·0	
	736	12	1·7	
	44	nil	0·0	Spontaneous arrest.
K. B. ..	3,200	1,224	38·0	
	216	30	14·0	Spontaneous arrest.
P. Y. ..	4,270	96	2·0	
	1,638	317	20·0	
	88	nil	0·0	
	60	nil	0·0	Spontaneous arrest
A. G. ..	1,297	160	12·0	
	1,100	320	30·0	
	336	30	9·0	Spontaneous arrest

On the average only about 20 per cent of young trophozoites reach maturity in the peripheral blood. Sometimes large crops of young trophozoites produce no schizonts whatever. This is seen towards the end of the fever undergoing spontaneous arrest. There are only two possible explanations of this phenomenon. One is that a steady destruction of parasites is taking place, and the other is that the parasites are disappearing into the internal organs as they mature. As to which is the true explanation we have no direct positive evidence. We can only argue on general lines. If as they mature the parasites disappear from the peripheral blood into the internal organs, the diminution will be most marked in the latter part of the apyrexial period and will affect the maturer forms only. We find, however, that the diminution occurs during the whole of the apyrexial period and affects all forms. Moreover a marked

diminution in the count is always followed by a diminution in the fever, and a disappearance of parasites by a cessation of the fever. This would not be so if the diminution was due to migration of parasites to the internal organs. In the literature of malaria we have found no evidence which proves that *P. vivax* disappears from the peripheral blood into the internal organs on approaching maturity. The present findings suggest that this may occur, but it cannot explain entirely the marked diminution in the parasites that is found in every case in the apyrexial period between the rigors. Almost certainly a marked destruction of parasites is going on.

(b) *P. falciparum*.

The usual finding is that after a rigor the counts fall markedly until the next rigor. In patient E. it fell from 66,000 to 360 and in patient I. B. it fell from 76,000 to less than 100. The forms that are found in the peripheral blood in the diminished counts are still early trophozoites, the result of a slight amount of schizogony still going on. Practically all parasites disappear from the peripheral blood after 24 hours development. To what is the disappearance due?

Here once more we have only indirect evidence. The fall in the count is not usually followed by diminution in fever and other symptoms at subsequent rigors. The reverse often takes place, a marked fall being often followed by hyperpyrexia at the next rigor. This disappearance of parasites is therefore due largely to the fact that the parasites as they mature go into the internal organs.

Is there any evidence that destruction of parasites may also contribute to this diminution in the peripheral blood? The present findings give us no evidence on this point. Other writers have recorded the finding of mature forms of *P. falciparum* ingested by phagocytic cells in the peripheral blood, but this is a rare finding and the destruction probably takes place in the internal organs.

E. THE BEARING OF THESE FINDINGS ON THE PROBLEM OF PARASITE DESTRUCTION.

We have found that in infections by the malarial plasmodia there is a tremendous destruction of parasites going on continually. This destruction is apparently of two kinds and two separate mechanisms may be at work.

Firstly there is a tremendous destruction of parasites in the free merozoite stage. Most of the merozoites never infect a red cell but are destroyed before they can do so. This phenomenon has long been recognised and there are many references to it in the literature but it has not previously been studied statistically. One important fact brought out in the present enquiry is that the destruction rate of free merozoites is comparatively low at the beginning of fever, and rises later so that nearly all free merozoites are destroyed and the fever subsides. The actual percentage of free merozoites destroyed apparently varies under differing circumstances from 50 per cent to 100 per cent.

Secondly there is a wholesale destruction of parasites not only in the free merozoite stage but at the later stages when the parasite is inside the red cell. This phenomenon occurs, and has been investigated, in bird malaria, but it has

only been suspected and not investigated in human malaria. Bass and Johns (1912) found that the greatest hazard to the parasite was at sporulation when it was passing from one red cell to another. This probably is true, but the present findings show that the parasites are frequently destroyed inside the red cell. Knowles, Acton and Das Gupta (1923) express the opinion that parasite destruction is confined to the free merozoite stage of the parasite and that once the parasite is inside the red cell it is safe until the next sporulation. We have seen that this is not so, that there is a wholesale destruction of parasites that have infected red cells, and that this is an important phenomenon in the spontaneous arrest of malaria.

Our results indicate that these two processes of parasite destruction are both active from the start of the fever but that, as fever continues, an acquired immunity is apparently developed, causing an increase in the destruction rate of the parasites and tending to produce spontaneous arrest.

Regarding the mechanism of parasite destruction our present findings tell us little. Apparently, free merozoites undergo lysis or phagocytosis. The destruction of parasites inside red cells can only take place by either lysis of the red cell followed by destruction of the liberated parasites, or else by ingestion of the whole red cell with the contained parasite by phagocytic cells. We have searched carefully in the hundreds of films examined in the present enquiry for any evidence of phagocytosis of parasites in the peripheral blood. All we have seen is the occurrence of malarial pigment inside large mononuclear cells. This finding is not uncommon and it does not prove that parasites have been ingested, for the pigment ingested may be merely that liberated at each completed schizogony by the bursting of the red cells. So we have no evidence from the present enquiry that phagocytosis of parasites takes place in the peripheral blood. This has however been recorded by Knowles and Das Gupta (1931), but it is obviously very rare. Millions of malarial parasites may disappear from the blood within a few hours, and, if phagocytosis in the peripheral blood were an important agency in producing this disappearance, it would surely have been more frequently observed. We therefore believe that the negative finding in the present series of cases is of importance, for it means that phagocytosis of parasites takes place almost entirely in the internal organs.

It has long been recognised that the reticulo-endothelial system is intimately connected with the immunology of malaria. If the present findings are true, it must be the fixed cells and not the wandering cells of this system which are most concerned with parasite destruction. Efforts in a few of our cases to obtain evidence of phagocytosis of parasites by the Kupffer cells of the liver gave negative results. It is not easy to get satisfactory liver puncture material.

We think, however, that the destruction of red cells infected by malarial parasites may be merely a development of the ordinary process of red cell destruction which goes on in the normal healthy body. Effete red cells are normally destroyed by ingestion by the cells of the reticulo-endothelial system, the most important of which in man are those of the spleen and bone marrow; it seems very likely that red cells infected by malarial parasites may be destroyed in the same way. We suggest that evidence on this point may be obtained by examination of the spleen and bone marrow in suitable cases. The finding of parasites ingested by the reticulo-endothelial cells of the spleen and

bone marrow has been made in post-mortem material. A series of spleen punctures in chronic malaria reported by Knowles, Acton and Das Gupta (1923) failed to show this phenomenon. Post-mortem material and chronic malarial spleens are not the best material in which to study the destruction of malarial parasites, since in the former the protective mechanism has broken down or death would not have occurred, and, in the latter, parasite destruction is comparatively slight. The best material is undoubtedly that obtained from fresh cases of malaria, but this is difficult to obtain.

SUMMARY.

1. A series of cases of malaria due to *P. vivax* and *P. falciparum* (mostly fresh infections) were investigated by means of four-hourly total and differential parasite counts, together with four-hourly temperature charts. Parasite counts up to 110,000 *P. vivax* and 202,000 *P. falciparum* per c.mm. of blood are recorded.

2. The principal factors influencing the parasite count are discussed and are enumerated as follow: (a) the species and strain of the Plasmodium, (b) various factors in the patient, such as age, previous malarial infection, intercurrent disease, and (c) the time of examination in relation to the time of the rigor and the duration of the fever.

3. Studies of the relation between the parasite count and the fever in malaria due to *P. vivax* give the following results:

- (a) A minimum parasite count of about 500 per c.mm. of blood is usually necessary to cause fever, but there were marked differences in different patients under different conditions.
- (b) The marked variations in the parasite count in a patient are due chiefly to multiplication and destruction of parasites, and only slightly if at all to migration of mature parasites to the internal organs.
- (c) In any one patient, increase or decrease in the parasite count is often accompanied by increase or decrease of the fever. In different patients, the same parasite count is often accompanied by marked differences in the degree of fever produced.
- (d) The schizont count before a rigor is compared with the young trophozoite count after the same rigor. The largest increase observed was a ninefold one. Usually the increase was much less than this; often there was actually a diminution, and sometimes no young trophozoites were found. Thus it is shown that, of the merozoites liberated after schizogony, a large number fail to infect red cells and are destroyed, the destruction rate varying between 50 per cent and 100 per cent.
- (e) The young trophozoite count after one rigor is compared with the schizont count about forty hours later. It is thus found that a large number of trophozoites fail to mature, as they are apparently destroyed. The percentage attaining maturity varies from 0 to 90 per cent and averages 20 per cent.

4. Studies of the relation between the parasite count and the fever in malaria due to *P. falciparum* gave the following results :

- (a) Estimation of the parasite count necessary to cause fever proved difficult. Such results as were obtained tend to support the findings of Ross and Thomson that from 600 to 1,500 parasites per c.mm. of blood are usually necessary.
- (b) Very marked variations in the parasite count within a short period are recorded. These variations are considered to be due largely to migration of mature parasites to, and of young parasites from, the internal organs.
- (c) Increase or decrease in the parasite count is not necessarily followed by an increase or a decrease in the fever, marked diminutions or even disappearance of the parasites from the peripheral blood being often followed in a few hours by hyperpyrexia with a tremendous increase in the young trophozoite count. The degree of fever is roughly proportional to the count soon after the rigor.
- (d) Owing to schizogony taking place almost entirely in the internal organs, it was not found possible to study numerically the phenomenon of multiplication and destruction of parasites.

5. The bearing of these findings on the problem of the mechanism of destruction of malarial parasites is discussed. Destruction is considered to occur in two ways: (a) lysis or phagocytosis of free merozoites, and (b) ingestion of infected red cells by the reticulo-endothelial system. Both these processes operate from the beginning of fever, but, as time goes on, these processes are augmented with often a spontaneous arrest of fever and disappearance of the parasites.

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NOTES ON MALARIA IN MYSORE STATE.*

Part VII.†

THE ANOPHELINE TRANSMITTERS OF MALARIA.

BY

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[3rd April, 1934.]

SWEET AND RAO (1931) published a report giving the results of anopheline dissections performed in three selected study stations in endemic malaria areas of Mysore State. The anophelines were caught in houses, cattle-sheds, and combinations of them, and were kept in lamp chimneys, usually for 72 hours, before dissection. In that report dissections of 31,277 anophelines were listed, and a report was made of eleven infections, of which three were stomach infections and eight gland infections. These infections were found in *A. culicifacies*, the *A. fluviatilis* group (*A. fluviatilis*‡, *A. minimus* and *A. varuna*), and in *A. stephensi*.

Part I of these notes gave a description of the three study stations and Part II discussed the anophelines of Mysore (Sweet, 1933a and 1933b). In Part II a short summary of the results of dissections was given and it was stated that further work would be reported later. This part reports dissections of anophelines subsequent to January, 1932, carried out in the three study stations, and in another area in which there was an epidemic occurrence of malaria.

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‡*A. fluviatilis* was formerly called *A. listoni*.

METHODS.

The anophelines here reported were caught in three situations: (a) in houses and cattle-sheds in endemic areas; (b) in a tent with human bait in endemic areas; (c) in houses and cattle-sheds in the Mandya area in Mysore District during an epidemic of malaria. The endemic areas were in or near the three study stations established in 1928 and 1929. These were (a) the Nagenhalli area in Mysore District, three miles from Mysore City; (b) the Mudigere area in Kadur District; and (c) the Hiriyur area in Chitaldrug District. Included in the Hiriyur area was the village of Marikanave, 12 miles from Hiriyur town (Sweet and Rao, 1933). Since malaria control work had been begun in all three stations, it was necessary to select either the villages of the peripheral zones or adjacent villages for the catching of anophelines for dissection. Catches in Marikanave were made subsequent to the stopping of the experimental control by weekly doses of plasmoquine compound. All the house and cattle-shed catches were made between 8 o'clock in the morning and noon. A total of 2,634 anophelines from these catches were dissected.

As another source of anopheline capture, a tent was used in the three endemic areas. The tent was a small one in which it was possible to reach all parts. The sides and back flaps were fitted close to the ground and earth was piled along the outside bottom edge to prevent egress; the front flaps were kept open, except for the time necessary to make catches. Two members of the staff, with a constantly lighted lantern, entered the tent at about 6 in the evening and made catches of all anophelines at about 9 p.m., midnight, 4 a.m., and 6 a.m. During the work here reported, 927 anophelines were caught in the tent. As a rule, the tent was set up in a vacant plot near houses in the selected villages.

In an area to the north and west of Mandya town (on the Bangalore-Mysore railway and road), irrigation began on lands under the new Irwin Canal in January, 1932, and by April of that year complaints of increased malarial prevalence began to be received by the health authorities. A pronounced epidemic was established by October and November of that year and, although the incidence of cases subsided somewhat between December, 1932 and February, 1933, there was again a smaller epidemic from March to June, 1933. Anophelines were caught in houses and cattle-sheds of this area between 8 a.m. and 12 noon, but the tent was not used.

The anophelines caught were kept in lamp chimneys for from 72 to 120 hours and were then dissected in the usual way. Examinations of the stomachs and glands were made by one of us (D. N.).

It is of interest to note that the members of the staff who made catches in the tent were given single daily doses of 0.1 gm., of atebirin and 0.01 gm. of plasmoquine, and that no cases of malaria resulted among them, although infected anophelines were caught and possibly others entered, fed, and left the tent between catching periods.

Visits were made to the Nagenhalli area in March and April, 1933, and again in November and December; to the Mudigere area in May, 1932, May, 1933, and October, 1933; and to the Hiriyur area in December, 1932 and November, 1933. The Mandya area was visited in October and November, 1932, April, 1933, and in August and September, 1933.

RESULTS OF DISSECTIONS.

DISSECTIONS IN ENDEMIC AREAS.

In the three endemic areas 3,438 anophelines, belonging to nineteen species, were dissected and 3,424 stomach and 3,403 gland preparations were examined. The incidence of stomach infections was 1·2 per cent and 0·2 per cent of the gland preparations showed sporozoites. In only one instance were both stomach and gland found infected at the same time, so that 1·4 per cent of the mosquitoes dissected showed signs of malaria infection. Table I gives the results of dissections carried out in all three of the endemic areas.

TABLE I.

Results of anopheline dissections performed in three endemic malaria areas in Mysore State in 1932 and 1933.

Species.	Number dissected	STOMACHS.			GLANDS			RESULTS.	
		Number examined.	Number with cysts.	Per cent.	Number examined.	Number with sporozoites.	Per cent.	Number of mosquitoes with signs of infection.	Per cent.
<i>aconitus</i> ..	123	120	0	..	121	0	..	0	..
<i>annularis</i> *	129	129	0	..	128	0	..	0	..
<i>barbirostris</i> ..	20	18	0	..	18	0	..	0	..
<i>culicifacies</i> ..	813	812	20	25	810	2	0·2	22	27
<i>fluvialis</i> *	657	656	16	24	648	5	0·8	20	30
<i>hyrcanus</i> †	166	164	0	..	166	0	..	0	..
<i>jamesi</i> ..	120	119	0	..	117	0	..	0	..
<i>jeyporiensis</i> ..	674	671	2	0·3	667	0	..	2	0·3
<i>karwari</i> ..	24	24	0	..	23	0	..	0	..
<i>leucosphyrus</i> ..	4	4	0	..	4	0	..	0	..
<i>maculatus</i> ..	1	1	0	..	1	0	..	0	..
<i>pallidus</i> ..	8	8	0	..	8	0	..	0	..
<i>splendidus</i> *	13	13	0	..	13	0	..	0	..
<i>stephensi</i> ..	278	277	3	1·1	278	0	..	3	1·1
<i>subpictus</i> ..	261	261	0	..	254	0	..	0	..
<i>tessellatus</i> ..	11	11	0	..	11	0	..	0	..
<i>turkhudi</i> ..	4	4	0	..	4	0	..	0	..
<i>vagus</i> ..	99	99	0	..	99	0	..	0	..
<i>varuna</i> ..	33	33	1	3·0	33	0	..	1	3·0
All species ..	3,438	3,424	42	1·2	3,403	7	0·2	48	1·4

* *A. annularis* was formerly known as *A. fuliginosus*; *A. splendidus* was formerly known as *A. maculipalpis* var. *indiensis*; and *A. fluvialis* as *A. listoni*.

† *A. hyrcanus* var. *nigerrimus* Giles, 1900

In *A. culicifacies* 2.5 per cent of the stomachs were found to show oöcysts and sporozoites were found in 0.2 per cent of the salivary gland preparations, while in *A. fluviatilis* corresponding percentages were 2.4 and 0.8. No gland infections were found in any other species, but 1.1 per cent of *A. stephensi* stomachs had oöcysts and 0.3 per cent of the *A. jeyporiensis* stomachs. One *A. varuna* stomach among 33 examined was found to have oöcysts.

Nagenhalli area.

In the Nagenhalli area, four *A. culicifacies* stomachs showed oöcysts during the March and April visit when 271 *A. culicifacies* were examined (1.5 per cent). No gland infections were found. Four stomach infections were found in 164 *A. fluviatilis* preparations examined (2.4 per cent), and one infection in 158 glands examined (0.6 per cent). These were all found during the November-December visit, and one *A. fluviatilis* had both stomach and glands infected. The only *A. varuna* stomach showing oöcysts was found in this area during the autumn visit, among 19 stomachs examined. Previously reported infected anophelines in this area were a stomach and a gland infection in *A. culicifacies* and one *A. fluviatilis* gland infection, all in April, with one *A. culicifacies* gland infection in August. It would seem that in this area *A. culicifacies* is the transmitter of malaria in the March to June season, and that *A. fluviatilis* has this rôle in the autumn months of the year. Despite the fact that *A. culicifacies* is most numerous from June to September, and that one gland infection was reported in August, the writers do not feel, from experience here and elsewhere in the State, that this period is even a moderately active transmission season. There seems to be no possibility, however, of avoiding the conclusion that *A. fluviatilis* is an important carrier of malaria in the period between October and December, when it also has its maximum incidence. As to *A. varuna*, it is only possible as yet to say that it is a rare mosquito in this area, as is *A. stephensi*. No infection was found in 75 *A. jeyporiensis* dissected in the Nagenhalli area, and this is also a rare species here, as compared with the Mudigere area.

Mudigere area.

There have been no previous reports of infected anophelines from the Mudigere area. The present work, however, yielded three stomach infections and two gland infections among 168 specimens of *A. fluviatilis* examined in May and two stomach infections in 503 *A. jeyporiensis* examined in October. There were no infections in 31 *A. culicifacies* examined. No *A. fluviatilis*, and only two *A. culicifacies*, were captured during the October visit to this area. *A. varuna* is a rare species in Mudigere and there was no infection in the 12 specimens examined. It was only in the fifth year of work, 1933, that *A. stephensi* was found in very small numbers, so it is not at all probable that this species is of importance. *A. jeyporiensis* is the preponderating species of the area and has its maximum incidence in the later months of the year, when the two stomach infections were found. There is no other evidence of malaria transmission at this time of the year, and it seems highly probable that these stomach infections were of little or no significance. It seems safe to conclude that the transmission season in this area is between March and July and that *A. fluviatilis* is the carrier, with the reservation that *A. culicifacies* may be of more importance than has as yet appeared to be the case.

Hiriyur area.

Unfortunately, during the work here reported, it was not found possible to visit the Hiriyur area in the period between March and June, so that later information than that given in the previous report (Sweet and Rao, 1931) is not available. That report listed two stomach infections in *A. stephensi* caught in September. From the November and December visits of 1932 and 1933 there were reported 16 stomach infections and two gland infections in 447 *A. culicifacies*, and 9 stomach infections and two gland infections in 322 specimens of *A. fluviatilis*. In addition, three stomach infections were found in 274 *A. stephensi*, but no gland infections. Both *A. jeyporiensis* and *A. varuna* are very rare in the Hiriyur area. It is possible that there is no effective transmission in the Hiriyur area until after the monsoon, which usually begins about the end of June, but further work is required between March and June before this can be definitely stated. There would seem to be no question, however, about a malaria transmission season in the later months of the year, for which *A. culicifacies* and *A. fluviatilis* are the carriers. *A. stephensi* may play a minor rôle.

DISSECTIONS DURING AN EPIDEMIC.

In the Mandya area during an epidemic of malaria, 1,683 anophelines were dissected in the course of three visits at different times of the year. The results of these dissections are given in Table II.

TABLE II.

Results of all dissections in 1932-33 in the Mandya area, under epidemic conditions.

Species.	Number dissected.	STOMACHS.		SALIVARY GLANDS.		MOSQUITOES.	
		Number with oöcysts.	Per cent.	Number with sporozoites.	Per cent.	Number infected.	Per cent.
<i>aconitus</i> ..	14	0	..	0	..	0	..
<i>annularis</i> ..	51	0	..	0	..	0	..
<i>culicifacies</i> ..	1,151	26	2·3	12	1·0	37	3·2
<i>fluviatilis</i> ..	21	0	..	0	..	0	..
<i>hyrcanus</i> ..	10	0	..	0	..	0	..
<i>jamesi</i> ..	7	0	..	0	..	0	..
<i>jeyporiensis</i> ..	2	0	..	0	..	0	..
<i>pallidus</i> ..	2	0	..	0	..	0	..
<i>stephensi</i> ..	238	0	..	0	..	0	..
<i>subpictus</i> ..	134	0	..	0	..	0	..
<i>tessellatus</i> ..	5	0	..	0	..	0	..
<i>turkhudi</i> ..	2	0	..	0	..	0	..
<i>vagus</i> ..	38	0	..	0	..	0	..
<i>varuna</i> ..	8	0	..	0	..	0	..
All species ..	1,683	26	1·5	12	0·7	37	2·2

No infections were found in any of the anopheline species dissected, with the exception of *A. culicifacies*. In this species there were 37 among 1,151 mosquitoes which showed signs of being infected, a total of 3.2 per cent. Unfortunately the catch of *A. fluviatilis* was too small to determine the rôle of this species as a carrier under these epidemic conditions. In each epidemic studied in Mysore there have been found an abnormally large number of *A. culicifacies*, and it is probable that such changes in natural conditions as will cause this excess production will produce an epidemic. There is no reason to suppose, however, that *A. fluviatilis* will not also carry malaria at such times, if present, although it may never be the main carrier under epidemic conditions.

During the October-November visit, 653 *A. culicifacies* were dissected, among which 16 stomachs were found to have oöcysts, and 10 glands sporozoites, one mosquito showing both stomach and glands infected. Of the 143 *A. culicifacies* dissected in April, five had stomach infections and two had gland infections, but in the August-September visit there were no gland infections and only five stomach infections among the 355 *A. culicifacies* examined. The infection rates for stomachs during the three visits were, respectively, 2.4, 3.5, and 1.4 per cent and the corresponding gland infection rates were 1.5, 1.4, and 0.0 per cent. Judged by the incidence of malaria cases, there were seasons of maximum occurrence from March to June and from October to the middle of December, with the earlier season showing, possibly, the greater number of cases; in the months between these two seasons there was a distinct falling off in the number of cases. The findings as to infected *A. culicifacies* confirm this occurrence except that there was little, if any, difference between the spring and autumn infection rates.

There was not as great a difference between the anopheline infection rates of the endemic and epidemic areas as might have been expected theoretically, the greater occurrence and severity of the disease in the epidemic being due to the fact that the inhabitants of this area were not protected by numerous previous malaria infections.

TENT COLLECTIONS AS COMPARED TO HOUSE AND CATTLE-SHED CATCHES.

The total catch of *A. culicifacies* and *A. fluviatilis* in the endemic areas was 1,470 and of these 42 mosquitoes showed either stomach or gland infection or both, an infection incidence of 2.9 per cent. Of the 1,470 anophelines of these two species, 369 were caught in the tent and had an infection incidence of 2.2 per cent, while in 1,101 mosquitoes caught in houses and in cattle-sheds the rate was 3.1 per cent. Apparently, no advantage was gained, so far as finding infection in mosquitoes was concerned, by the use of the tent with human bait. It is possible that night collections in houses and cattle-sheds would show higher sporozoite rates in mosquitoes than did the morning collections, but this comparison was not attempted on account of the difficulty of gaining admission to houses and cattle-sheds during the night.

Certain other aspects of the tent collections of anophelines, and their relations to the house and cattle-shed collections, are of interest in a consideration of some of the habits of anophelines in Mysore. Table III gives data

in regard to the time of catching in the tent of nine anopheline species, and compares the total tent collection of these species with the house and cattle-shed collection. The total tent catch of these nine species was 773, of which 47 per cent were caught at 9 p.m. and midnight, and 53 per cent were caught at 4 and 6 a.m. Many houses and cattle-sheds were searched for anophelines every morning but only one tent was used at night, so the total catch in houses and cattle-sheds was considerably larger than in the tent.

TABLE III.

Tent catches of nine species of anophelines during two parts of the night and comparison of tent catches with house and cattle-shed catches.

Species.	CAUGHT IN TENT AT 9 P.M. AND MIDNIGHT.		CAUGHT IN TENT AT 4 AND 6 A.M.		TENT COLLECTION		HOUSE AND CATTLE- SHED CATCH.	
	Num- ber.	Per cent of tent catch of species.	Num- ber.	Per cent of tent catch of species	Num- ber.	Per cent of total tent catch.	Num- ber.	Per cent of total house and cattle- shed catch.
<i>aconitus</i> ..	2	13.0	13	87.0	15	1.9±0.3	111	4.8±0.3
<i>annularis</i> ..	14	58.0	10	42.0	24	3.1±0.4	111	4.8±0.3
<i>culicifacies</i> ..	48	33.0	96	67.0	144	18.6±0.9	686	29.7±0.6
<i>fluvialis</i> ..	119	53.0	106	47.0	225	29.2±1.1	440	19.0±0.5
<i>hyrcanus</i> ..	79	59.0	55	41.0	134	17.3±0.9	64	2.8±0.2
<i>jamesi</i> ..	26	55.0	21	45.0	47	6.1±0.6	81	3.5±0.3
<i>jeyporiensis</i> ..	60	46.0	71	54.0	131	16.9±0.9	551	23.8±0.6
<i>stephensi</i> ..	16	33.0	32	67.0	48	6.2±0.6	240	10.4±0.4
<i>varuna</i> ..	2	40.0	3	60.0	5	0.6±0.2	28	1.2±0.1
All species ..	366	47.0	407	53.0	773	100.0	2,312	100.0

Catches of *A. aconitus*, *A. culicifacies*, *A. jeyporiensis*, and *A. stephensi* constituted a significantly higher percentage of the morning house and cattle-shed catches than of the night tent collections. These species apparently have a decided preference for habitations for daytime resting places, and catches made in houses and cattle-sheds will not give an underestimate of the prevalence of these species in any area. In the case of *A. annularis* and *A. varuna* there appeared to be the same tendency, but it was not so marked as in the first-mentioned species.

The catch of *A. varuna* was very small and was included only because oöcyts were found in the stomach of one specimen; it would seem from our meagre catch that *A. varuna* differs from *A. fluvialis* in its choice of a resting place.

The *A. stephensi* caught were mainly from the Hiriyur area and have not the extremely domestic habits of urban specimens of this species. In that area *A. stephensi* breeds in the open in swamps and reedy streams and is a wild species; it still, however, prefers habitations as daytime resting places.

A. fluviatilis, *A. hyrcanus*, and *A. jamesi* were decidedly more common in the tent catches than in the house and cattle-shed collections. These species enter habitations for feeding but, apparently, prefer to leave them for other daytime resting places. Catches of these species in houses and cattle-sheds during the daytime hours will give too low an estimate of their prevalence.

It is of interest to note that *A. culicifacies* constituted a somewhat higher proportion of the tent catches than of the house and cattle-shed catches in the Nagenhalli and Mudigere areas, but was nearing 50 per cent of the Hiriyur house and cattle-shed catches. *A. fluviatilis* was more common in tent catches in Hiriyur and very markedly more common in Mudigere, but the situation was reversed in Nagenhalli where it was slightly more common in house catches than in tent catches.

In recent similar studies in Savantvadi State, 65 miles west of Belgaum and below the ghats, the catch of *A. fluviatilis* was 50 per cent of the tent collection and less than one per cent of the house and cattle-shed collection, while corresponding percentages for *A. culicifacies* catches were 36.0 and 18.0. There is, then, local variation in the habits of these two species. Is it possible that these differences are due to the presence of different proportions of two or more varieties of these species, as is the case with *A. maculipennis* in Europe?

With the exception of *A. annularis*, well over 50 per cent of the species of anophelines which were more common in house and cattle-shed catches, were caught in the tent during the latter part of the night, and the reverse was true of those species more common in the tent catches. For instance, 67 per cent of the total tent catch of *A. culicifacies* was caught at 4 and 6 a.m., while 53 per cent of the *A. fluviatilis* catch was made at 9 p.m. and midnight. Species which seem to prefer outside resting places would naturally be moving in search of food during the earlier part of the night, and, since a certain number of these species do rest in houses and cattle-sheds, they would probably have a smaller number in flight in the early morning hours, after feeding. In the case of the other species, an outside flight for food would not be necessary, and the heavier early morning catch possibly represents flight for oviposition and change of resting places for other reasons.

SUMMARY.

Part VII of the Notes on Malaria in Mysore State reports on dissections of anophelines caught at night in a tent with human bait, and in houses and cattle-sheds of three areas of the State in which malaria is endemic; also in houses and cattle-sheds of an area in which there was an epidemic of malaria. In the endemic areas the oöcyst rate in *A. culicifacies* was 2.5 per cent and the sporozoite rate was 0.2 per cent; corresponding rates for *A. fluviatilis* were 2.4 and 0.8 per cent. In addition, *A. jeyporiensis* had an oöcyst rate of 0.3 per cent and *A. stephensi* one of 1.1 per cent; one stomach among 33 *A. varuna* examined showed oöcysts. From the epidemic area, infections were reported from *A. culicifacies* only, the oöcyst rate being 2.3 per cent and the sporozoite rate 1.0 per cent. The catch of *A. fluviatilis* in this area was small. Apparently *A. culicifacies* and *A. fluviatilis* are the important malaria transmitters of the rural areas of Mysore; *A. stephensi* and *A. varuna* may

be minor carriers, but it was concluded that *A. jeyporiensis* was of no importance in this respect.

The infection rate of *A. culicifacies* and *A. fluviatilis* caught in the tent was 2.2 per cent, and these same species collected in houses and cattle-sheds had a corresponding rate of 3.1 per cent. It is suggested that night collections in houses and cattle-sheds might show higher sporozoite rates.

A. aconitus, *A. culicifacies*, *A. jeyporiensis*, and *A. stephensi* were in a higher proportion in house and cattle-shed collections than in tent catches, while the reverse was true of *A. fluviatilis*, *A. hyrcanus*, and *A. jamesi*. The first group would seem to use habitations for daytime resting places, but the second group seems to prefer other situations. More than half the tent catch of the four species of the first group was made at 4 and 6 a.m., but members of the three species of the second group were more commonly caught during the 9 p.m. and midnight collections.

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LARVAL SURVEY OF THE LAND AROUND BIRNAGAR AND DETERMINATION OF THE LONGEVITY OF THE LOCAL *ANOPHELES CULICIFACIES* AND ITS HABITS.

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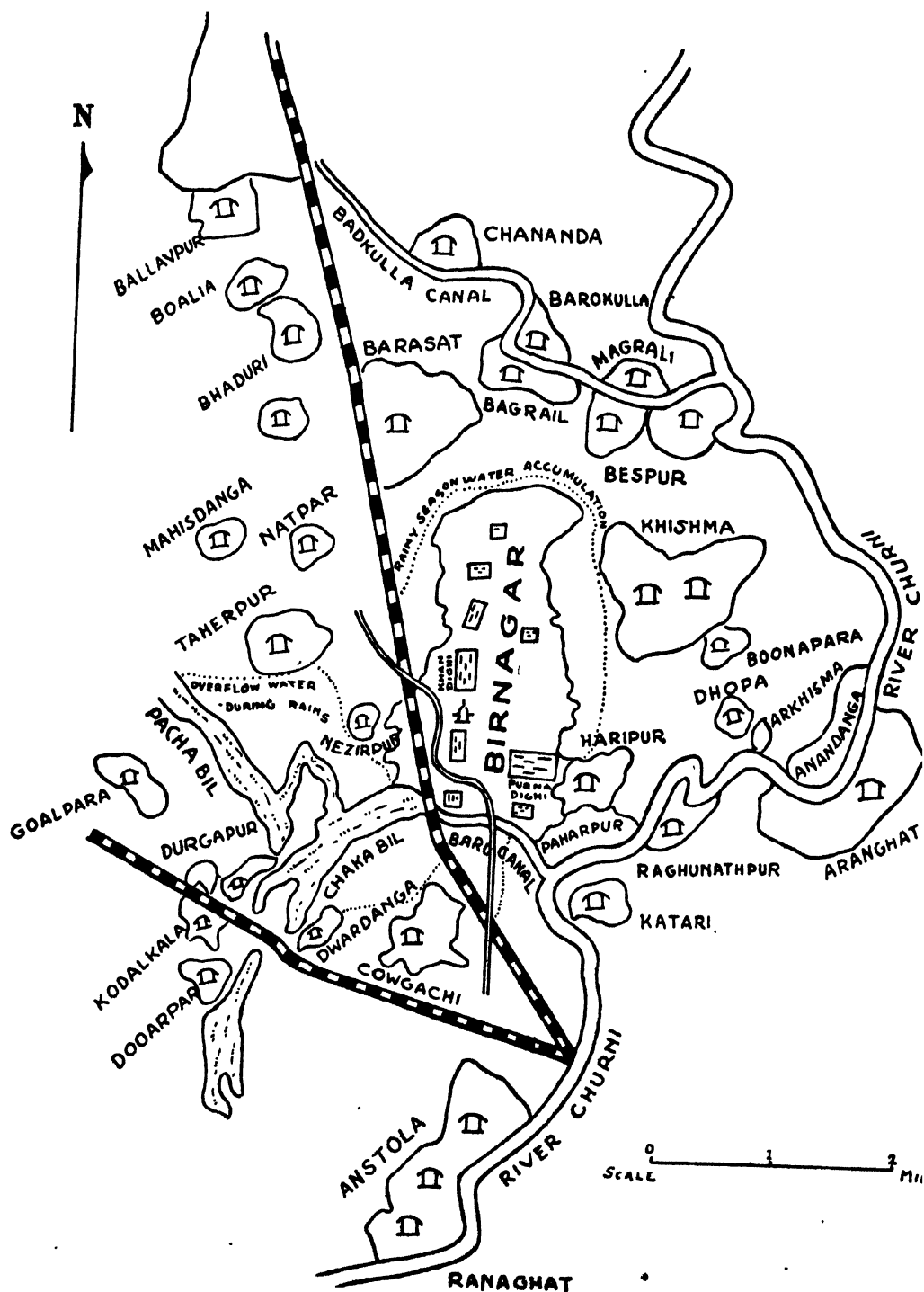
[14th April, 1934.]

INTRODUCTION.

THE researches conducted by Dr. P. Sur and the Birnagar Palli Mandali show that out of 12 Anopheline species present at Birnagar, *A. philippinensis* is the principal and practically the only dangerous anopheline to which our attention should be directed. Although our anti-larval operations were extended to the Baromesia Canal and the Chaka Bil, which lie outside Birnagar, much of our good work was spoilt by the influx of mosquitoes of the species named above from other quarters. A larval survey outside the municipal limits up to a radius of one mile or more was, therefore, carried out by the Mandali in 1932. As certain discrepancies were noticed between our findings and those of malariologists in other parts of India in regard to the behaviour of *Anopheles culicifacies*, which breeds in the River Churni, a re-survey of the area, particularly of the lands on both sides of that river for some distance, was undertaken during September and October 1933, in order to confirm our previous year's findings. The total area surveyed outside Birnagar, including the additional lands along the Churni, was about 15 square miles.

BREEDING PLACES OF ANOPHELINES.

There are rice-fields all around Birnagar, interspersed with villages, of which the population is mainly agricultural. The principal villages in the area investigated are Radhanagar, Shambhunagar, Paharpur, Haripur, Raghunathpur, Khisma, Bampur, Bagrail, Chananda, Barasat, Nazirpur, Cowgachi and Anstala. Of these Khisma, which was once opulent and



Birnagar and its environments.

prosperous, is in a state of ruin. The River Churni flows mostly three miles to the east of Birnagar, but takes a sharp turn towards Birnagar at the south-east, lessening the distance to half a mile for about a mile of its course. The Baromesia Canal, which forms the southern boundary of Birnagar, connects the Chaka Bil on the south-west with the Churni on the south-east. The Badkulla Canal, which issues from the Churni, flows a mile off to the north of Birnagar. This canal ends abruptly near Krishnagar. There are some large depressions, of which a few form part of the canal system, where water accumulates in the rains forming *bils*, e.g., Dakatyr Khal. These remain dry during the late winter and the summer seasons. There are *dighis* at Khisma and Barasat which are as large as the Khan Dighi at Birnagar. There are several tanks in Khisma and some at Cowgachi. There are numerous temporary *dobas* or pools in all the villages. Road-side excavations exist all along the Berhampore Road. There are numerous borrow-pits on both sides of the railway line on the southern and western side of Birnagar.

DISCUSSION OF IMPORTANCE OF ANOPHELINES FOUND IN VARIOUS BREEDING PLACES WITH SPECIAL REFERENCE TO *A. CULICIFACIES*.

THE CHURNI.

During the summer and winter seasons of 1932 and 1933 larvæ of the following species were found: *A. annularis*, *A. hyrcanus* var. *nigerrimus*, *A. culicifacies*, *A. barbirostris*, *A. vagus* and *A. subpictus*. Of these, we need confine our attention only to *A. culicifacies*, which is a dangerous carrier of malaria in the Punjab and in Southern India. We should be very careful in estimating its power of doing mischief in the Nadia district. The breeding of this species in the area investigated is confined to the Churni in the dry season, when malaria is not much in evidence. The Churni is in flood in the rainy season when breeding of mosquitoes is absent. We could not find *A. culicifacies* larvæ in any water collections in the rainy season in the villages washed by the Churni during 1932 and 1933. At Krishnagar this species breeds in the Jelanghi in the dry season. We have not made any mosquito collection from the villages on the Churni; but from the records of collection made by Dr. P. Sur from rural areas adjoining the Jelanghi (Sur and Sur, 1928), we find that the largest number of collection of mosquitoes of this species was made during the summer months. During the rains and the fever season they became scarce. They again appeared in the winter months. It is very strange that during the rainy season when all other Anopheline species become active and multiply, *A. culicifacies* should not only refrain from breeding but apparently keep itself in hiding. Sur did not find any malarial infection in *A. culicifacies* among the specimens dissected by him at Krishnagar during the years 1927 to 1932. The habits of breeding of this species in the Churni and the Jelanghi being the same, we may infer that it behaves similarly in other respects in the villages situated on those rivers.

Let us compare our findings with those made by Senior White (1930) at Delhi in regard to *A. culicifacies*. This species makes its appearance at Delhi generally after the onset of the rains when malaria breaks out. The Jumna rises late in July and floods the Bela, causing interference with the outfalls

of the various channels which drain into it. *A. culicifacies* breeds in these channels, and in the blocked drains. It has also been found to breed in the canal and temporary rain-pools and borrow-pits. It will thus be seen that mosquitoes of this species behave very differently in the two different regions, viz., the Nadia district and Delhi, and we may conclude that Nature has made them incapable to do any harm in the former area.

It however appears from Dr. Strickland's rapid larval survey of the districts of Bengal made in 1926-27 (*vide* Strickland and Chowdhury, 1927) that *culicifacies* breed not only in streams but also in other kinds of water collections in the districts of Murshidabad, Burdwan and Birbhum. We thus find that in Bengal the habits of breeding of *A. culicifacies* differ at a distance of 40 miles, say at Birnagar and Burdwan. But we have no information about the infectivity of this species prevalent in the three districts named above. Christophers (1933) expresses his opinion on the relation of this species to disease as follows: 'The most important malaria-carrying species in India, except perhaps in the eastern areas'.

Ordinarily the Churni rises at the end of July and the flood subsides in the first week of November. During this period the breeding of mosquitoes altogether ceases. As we have not found any *A. culicifacies* breeding within 3 miles on the Birnagar side and half a mile on the east side of the river during the rainy seasons of 1932 and 1933 along 5 miles of its course, we may safely conclude that these mosquitoes maintained their existence during the period the flood lasted. In 1933 the flood came early in July and subsided about the middle of November. Consequently the duration of the cessation of breeding was a little over four months, which must be regarded as the minimum period of longevity of *A. culicifacies*.

A. culicifacies is not a local species at Birnagar, but a few stray mosquitoes and a few larvæ were on rare occasions collected in that town. Apparently some mosquitoes are carried to Birnagar from the Churni area by carts and other kinds of conveyance, but they cannot thrive and die out, the place being obviously unsuitable for their breeding. As the shortest distance between Birnagar and the Churni is half a mile, we may conclude that *A. culicifacies* cannot ordinarily fly more than half a mile from its breeding place.

BILS.

There are two bils on the south-west of Birnagar called Pacha Bil and Chaka Bil. These are permanent water collections. The first one is situated at a distance of more than a mile from Birnagar and was not included in the scope of our enquiry. The Chaka Bil is one and a half miles long and has been found to be the largest breeding ground of *A. philippinensis*. Eight other species were also found to breed in this large sheet of water, namely, *A. annularis*, *A. pallidus*, *A. ramsayi*, *A. subpictus*, *A. vagus*, *A. hyrcanus* var. *nigerrimus*, *A. aconitus* and *A. barbirostris*. By dealing with this bil we hope to be able not only to reduce the incidence of malaria but also to control the general mosquito nuisance in a large measure. In the course of our larval survey in this bil we incidentally determined the flying capacity of *A. aconitus* to some extent. In the winter of 1930-31 we found adults of this species in certain cowsheds at Birnagar in large numbers, and their only breeding place in the neighbourhood was in the grassy edges of the eastern end of Chaka Bil.

As the distance between the cowsheds and Chaka Bil was one-third of a mile, we may conclude that mosquitoes of this species can easily travel such a distance in search of food. We are unable to say whether they can fly a longer distance.

BAROMESIA CANAL.

Larvæ of *A. annularis*, *A. aconitus* and *A. hyrcanus* var. *nigerrimus* were found. Only a few *philippinensis* larvæ were collected. Breeding of mosquitoes in this canal stops ordinarily during August owing to the influx of flood water from the River Churni, but the river-silt settles down in September, when mosquitoes recommence to breed.

BADKULLA CANAL.

This canal could not be examined during the rainy season and autumn, but, from a survey made during January 1933 for a distance of three miles from its junction with the Churni, we found that it contained mainly *A. hyrcanus* var. *nigerrimus*, *A. annularis* and *A. pallidus*. It has been ascertained from local enquiry that the silt-laden flood water of the Churni remains in this canal for a longer time than in the Baromesia Canal. It is unlikely that this canal is causing any harm to the locality.

TANKS AND DOBAS (POOLS).

These two kinds of water collections differ from each other ordinarily in depth and size. The *dobas*, which are temporary pools, were mostly formed by removing earth for building huts, or for stocking manure by the cultivators. The former class of *dobas* has generally clean bottoms. They do not seem to favour the growth of as much vegetation as the tanks, possibly because of their shallow depth. The species found to breed in such *dobas* are *A. subpictus*, *A. vagus*, *A. hyrcanus* var. *nigerrimus* and *A. annularis*, whereas larvæ of the first two species only were found in the manure pits. At Birnagar, however, we found *philippinensis* larvæ in a small number of *dobas* and in certain tank-like hollows, known as *baroj dobas*. Unlike the other pools, these *dobas* become full of water-weeds in the rainy season.

The tanks favour the breeding of all kinds of Anopheline species, except *A. culicifacies*. *A. philippinensis* was found to breed in almost all the tanks examined. Our investigations clearly show that it is essentially a tank-breeder so far as Birnagar and its environments are concerned. The bils, the *dighis*, and the tanks, though they differ in size, have almost similar depths and the same kinds of aquatic plants grow in them. In Chaka Bil there are, in addition, floating grasses, which invite *A. aconitus* and *A. ramsayi* to breed at their edges. Chaka Bil is the largest breeding ground of *A. philippinensis* in the area investigated, but the number of *philippinensis* larvæ found per square yard of water surface was much higher in *dighis* and tanks than in that bil. This species does not like to breed in open water surfaces and, in two of the clean tanks at Birnagar, we attracted them by introducing certain kinds of vegetation and repelled them by removing the same vegetation. Unfortunately most of the tanks at Birnagar and in the neighbouring villages are full of vegetation, which has its origin in bulbous and fibrous roots in their beds. These tanks can only be cleaned permanently by re-excavation, which is a costly affair. Even when tanks are re-excavated *A. philippinensis*

recommences to breed in them as soon as certain kinds of vegetation reappear. The Birnagar Municipality re-excavated Natunpokur in 1924 and Bagdipokur in 1926. *A. philippinensis* larvæ were first found in them after their re-excavation in 1930 and 1931, respectively, simultaneously with the reappearance of vegetation. Formerly Birnagar and its neighbouring villages contained a large population, but with their decadence since the malarial outbreak in 1856, most of the tanks have been left in a disused state. Christophers (1933) writes: 'Different species may show predilection for certain types of breeding place (selective breeding). This predilection is not always according to the actual type of breeding place, but depends upon some requirements that may be present in several types. ...there exist many oecological studies of breeding places dealing with favourable and unfavourable vegetation'.

RICE-FIELDS.

A. hyrcanus var. *nigerrimus* was the commonest species found breeding in large sheets of shallow water in the rice-fields. *A. annularis*, *A. barbirostris*, and in one instance *A. pallidus*, larvæ were also found. No *philippinensis* larvæ could be collected in such water areas.

BORROW-PITS.

The pits by the side of the Berhampore Road were mostly under shades of trees and contained blackish or brownish water, while those on both sides of the railway line were exposed to the sun and generally had clear water. But this made little difference in the species of larvæ found which were *A. hyrcanus* var. *nigerrimus*, *A. annularis*, *A. subpictus* and *A. vagus*. *A. philippinensis* larvæ were found only in a small number of railway borrow-pits.

From a review of the larval collections made during 1932 and 1933 in rural areas adjoining Birnagar, we find that *A. hyrcanus* var. *nigerrimus* is the commonest species breeding in the locality. *A. annularis* comes next, then come *A. subpictus* and *A. vagus*. The breeding of *A. philippinensis* is confined to Chaka Bil, Dakatyr Khal (a temporary bil) and all *dighis* and most of the tanks examined. I have already shown (Bose, 1931) that, so far as Birnagar is concerned, we have little to fear from any Anopheline species other than *A. philippinensis*. Having ruled out *A. culicifacies*, the only new species prevalent in the Churni area, as a carrier of malaria, and the Anopheline fauna in other respects in rural areas and at Birnagar being the same, we may conclude that *A. philippinensis* is also the principal carrier of malaria in the villages around Birnagar.

SUMMARY.

The larval survey carried out in the villages around Birnagar during 1932 and 1933 shows that the Anopheline fauna of the locality is similar to that of Birnagar, except that *A. culicifacies* was found to breed in the Churni river in the non-flood season. The survey throws light on the habits and longevity of the local strain of this species, and points to *A. philippinensis* as the principal carrier of malaria in this locality, as it is at Birnagar.

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STUDIES ON THE LONGEVITY OF SOME INDIAN ANOPHELINES.

Part I.

SURVIVAL OF *ANOPHELES SUBPINCTUS* GRASSI UNDER CONTROLLED CONDITIONS OF TEMPERATURE AND HUMIDITY.

BY

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INTRODUCTION.

It is now well recognized that the conditions affecting the longevity of anopheline mosquitoes have a very important bearing on the carriage of malaria. The work of Gill (1921, 1921a, 1925) has shown that under varying climatic conditions, chiefly related to humidity, the insects may not survive long enough to allow the development of the malaria parasite to the infective stage in their bodies. It is, therefore, essential from the point of view of the duration of anti-mosquito measures, to determine the climatic conditions under which such insects are likely to be dangerous. Such conditions may not be the same in every species of anophelines, so the present experiments were made to obtain more definite knowledge of these conditions.

The species which have been studied are *Anopheles subpictus* (*A. rossii*), *Anopheles annularis* (*A. fuliginosus*), and *Anopheles culicifacies*, and the results obtained with each of these are recorded in this series of papers.

The experiments were conducted at the Ross Field Experimental Station for Malaria at Karnal, with a Fellowship grant from the Rockefeller Foundation in the International Health Division, which is gratefully acknowledged. My sincere thanks are due to Lieut.-Col. J. A. Sinton, V.C., M.D., I.M.S.,

Director of the Malaria Survey of India, for necessary guidance and very valuable advice during the course of this work. He has also very kindly furnished me with several useful references incorporated in this paper. To the Indian Research Fund Association, I wish to express my thanks for giving me every laboratory facility through the Malaria Survey of India.

GENERAL CONSIDERATIONS ON *ANOPHELES SUBPICTUS* IN RELATION TO MALARIA.

Recent researches have shown that certain species of anophelines are either poor 'carriers' of human malaria or have never been found infected in nature. In an exhaustive summary of this subject Covell (1931) has reported that in nature *Anopheles subpictus* Grassi, has never been found to show infections in the very large numbers which have been dissected in different parts of India, Ceylon, and the Philippines, except on one occasion (gut only). Natural infections have been reported, however, by several observers in the Dutch East Indies.

In 1925, Gill proceeded to examine the real status of *Anopheles subpictus* (*rossii*) in relation to the transmission of human malaria. After an analysis of the various factors concerned, he came to the conclusion that the problem remained unsolved. At the same time he emphasized that, in the absence of direct proof based upon the discovery of infected adults, it would appear necessary that the insect must fulfil a number of conditions before it can be considered a natural carrier. Among other factors, he believed that the environmental conditions must be conducive to the active metabolism of the insect and its relatively prolonged life.

Conditions influencing environmental resistance, as defined by Chapman (1925), include factors such as parasitism and lack of food, also all of the other factors usually classified under physical autecology. The question, therefore, arises whether it is either of the two first-named factors or the physical conditions in the environment which affects the longevity of *Anopheles subpictus*.

High parasitization of the imagoes is unknown in this mosquito. Sinton (1932), while reviewing the work on helminthic infections in Indian anopheline mosquitoes, has referred to the findings, by Stephens and Christophers (1902), of encysted bodies, like 'flukes', in the adults of *Anopheles subpictus* (*rossii*). Iyengar (1929) also found immature forms of the Nematode *Mermis* in this mosquito. Although it has been demonstrated that larval nematodes have a markedly injurious effect on the insects they infest, there is hardly any evidence to show that the percentage of parasitization in this species has been high enough to account for a high mortality amongst the imagoes.

Regarding the food factor, it is well known that the females have a great avidity for blood and consequently they have an ample food supply amongst human beings and cattle.

It is very likely that the physical factors, more particularly, temperature and humidity, might have some pronounced effect on the duration of life of these insects. The imagoes possibly cannot survive long enough for the

sporozoites to develop, and thus the mosquitoes will not be able to transmit malaria.

Existing literature on the subject is very fragmentary and there is also lack of properly controlled experimental work. It has been pointed out by Gill (1925) that this insect is highly susceptible to low degrees of relative humidity. In the absence of a prolonged series of experiments, he stated it was not safe to conclude whether or no atmospheric conditions play any appreciable part in restricting the power of *Anopheles rossii* to carry malaria in nature. According to him, the imagines must be able to survive for at least 12 days after imbibing infected blood before they can play any part in the spread of malaria.

Mayne (1930) conducted some experiments on the effect of atmospheric humidity on the longevity of *Anopheles subpictus* and found that low humidities were detrimental to the life of this insect. Judging from his results, the technique used by this worker would, however, appear to require verification by more precise ecological methods and with this object in view the present research has been undertaken.

Senior-White has cited observations (unpublished report) from the experiments conducted by Parasarathy that in hot weather no degree of saturation will prolong the life of *Anopheles rossii* for more than five days, though quite ordinary percentages give it a long life in cold weather. On this assumption, he suggested that this may be the reason why *Anopheles rossii* is a 'non-carrier' in nature, though susceptible to laboratory infection as shown by Gill (1925).

The theory that the non-dangerous character of *Anopheles subpictus* may be due to the short period of its life under conditions of temperature suitable for malaria carriage appears hopeful. However, this idea as the only solution does not appear to be supported by the facts available. If it were purely a question of longevity, the early stages of the malaria parasite (oöcysts) should develop in *A. rossii* even if it should live for five days only, but as noted above the records of oöcysts in this species in nature are negligible.

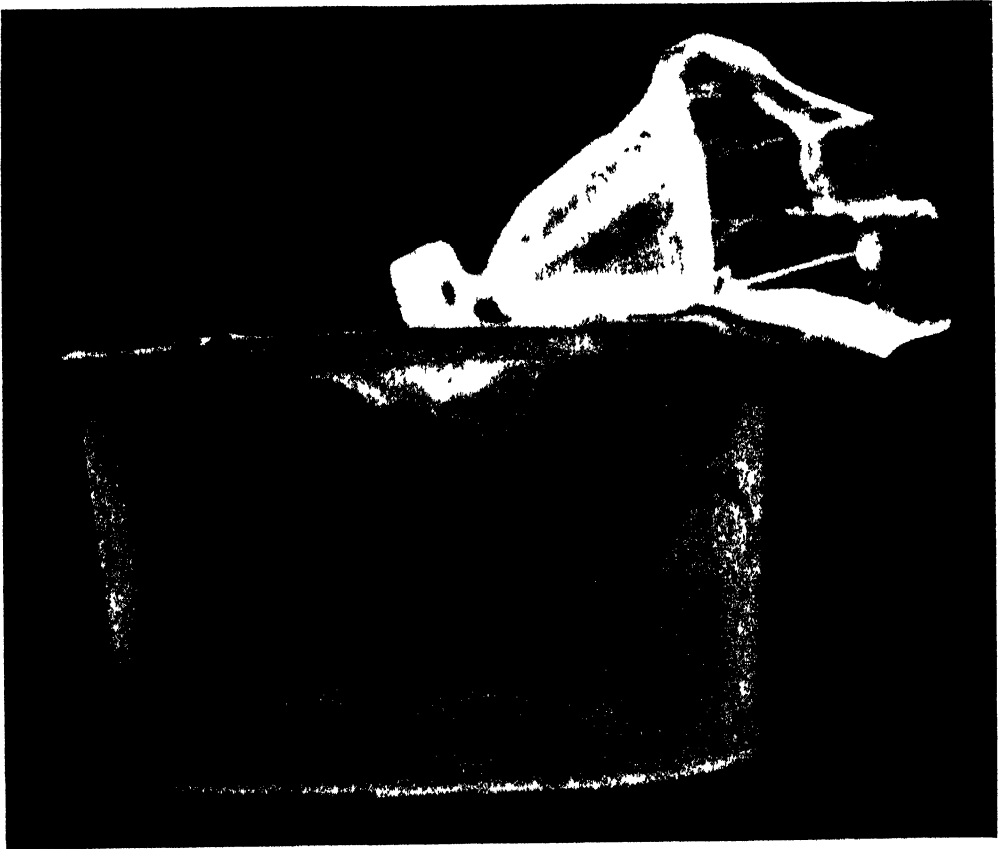
In the absence of any systematic experiments on these lines, it would perhaps be useful to elucidate how far these two factors—temperature and humidity—react on the longevity of the imagines. Any positive correlation might incidently show whether *Anopheles subpictus* (*rossii*) can live long enough for the completion of the sporogonous cycle of the malarial parasite. With this end in view, I have conducted several experiments on adults of known age obtained by hatching out from pupæ. Later these were given a blood meal and then exposed to known conditions of temperature and atmospheric humidity.

TECHNIQUE.

(a) APPARATUS.

The adults of *Anopheles subpictus* were hatched out in the laboratory from larvæ and pupæ collected in the field. The freshly emerged imagines

were then transferred to a cage made of ordinary mosquito-netting. They were given a blood meal about 10 to 12 hours after emergence. This was done by introducing one arm through a sleeve at one end of the cage, so that the mosquitoes could feed under observation. After the adults had become well engorged, they were transferred to a small spherical cage made of netting (as shown in the text-figure) through the sleeve 'S'. This cage has been designed to fit into the desiccators in which the humidity has been controlled with a sulphuric acid and water mixture.



On the roof of this cage 3 to 5 raisins (previously soaked in water till they became swollen and dried on a blotting paper), were placed for the adults to feed upon during the course of the experiments. It was found essential to soak the raisins in water so that the juice they contained got diluted to facilitate its passage through the proboscis of the mosquito. At the same time it could supply adequate amount of moisture for the existence of these insects when used in this manner. Experience has shown that when dry raisins were used, the mosquitoes failed to get a proper feed on account of the high viscosity of the juice and eventually died in a comparatively very short period. After preliminary experiments, it was ascertained that three to five raisins kept in

this manner did not very appreciably affect the relative humidity controlled within the desiccator. The raisins were changed daily to prevent any fungal development particularly when a low temperature of 25°C. (77°F.) was combined with a high humidity ranging from 70 to 90 per cent. In this way the experiments worked out very satisfactorily and the insects apparently lived their normal life.

(b) METHOD FOR THE CONTROL OF RELATIVE HUMIDITY.

The method for the control of humidity in the following experiments is essentially the same as has been previously employed in my earlier work on *Dysdercus cingulatus* Fabr. (Mehta, 1930).

The sulphuric-acid method for the control of relative humidity in the desiccators works out very effectively. Pure sulphuric acid (E. Merck's) mixed with distilled water was used in varying dilutions, and the corresponding percentages of relative humidity were computed. For this purpose the specific gravity of the mixture was determined with a set of Twaddle's hydrometers, and the figures thus obtained enabled one to work out the relative humidity by referring to Landolt Bornstein Roth's 'Physikalisch-Chemische Tabellen'. To verify the results thus obtained, a hair hygrometer was placed in the desiccator and no mosquitoes were kept in it till the humidity had settled down to the required percentage. If it were found that the humidity had altered during the course of the experiment, more sulphuric acid or water was added accordingly, and the desired density of the acid and water mixture was thus attained.

It has been pointed out by Knowles and his collaborators (1932) that the sulphuric-acid method did not work out satisfactorily in their experiments on account of the acid vapours in the closed desiccators. Moreover they believe that the humidity just above the surface of the acid solution may be entirely different from that in the upper layers of the chamber.

Regarding the first objection, it is difficult to imagine why this defect should occur unless pure sulphuric acid was not used, since impure acid is liable to give off the fumes. In my experiments both on the red cotton bug *Dysdercus cingulatus* Fabr. (Mehta, 1930) and the mosquitoes, there had been no such trouble as the acid used was pure.

As for the second objection, I have carefully tested for this possible fallacy and have been convinced that the humidity just above the surface of the acid solution is the same as in the upper layers of the chamber, since the acid mixture works on a restricted amount of air contained in the desiccator. It is quite possible that this difference in humidity may take place when the acid and water mixture is just transferred to the chamber. If, however, it were allowed to remain for some time with the lid of the desiccator kept firmly on the top, it gave a uniform humidity which could be tested with a 'Precision hair hygrometer'.

METHODS OF CONTROL OF TEMPERATURE.

Hearson's oil and electric incubators were used for the control of temperature. In summer, when the room temperature rose to 100°F. or more,

the experiments at lower temperatures were carried out in Hearson's cooling incubators.

EXPERIMENTAL RESULTS.

The object of the experiments was to determine the influence of temperature and atmospheric humidity on the longevity of the adult mosquito.

Freshly hatched imagines of *Anopheles subpictus* were given a blood meal about 10 to 12 hours after emergence, and were then confined in desiccators with varying percentages of relative humidity.

In the first series of experiments a temperature of 40°C. (104°F.) was kept constant, and hatches of 36 females of known age were confined in the desiccators at 50 per cent and 90 per cent relative humidity respectively. It was found that the maximum duration of life of these insects under these conditions did not exceed 50 hours, and fifty per cent of them did not survive for more than 24 hours.

At a temperature of 35°C. (95°F.), when kept in the desiccators at relative humidity varying from 30 per cent to 90 per cent, the longevity of these insects was very much prolonged. The details of these experiments have been incorporated in Table I and also graphically represented in Chart I.

TABLE I.
Constant temperature 35°C. (95°F.).

Experiment number.	Number of females.	Relative humidity, per cent.	Date adults emerged.	Date of blood meal.	50 per cent mortality in days.	75 per cent mortality in days.
1	21	30	29-7-33	30-7-33.	3	4
2	30	50	31-7-33	1-8-33	6	7
3	30	70	28-7-33	29-7-33	7	8
4	30	90	31-7-33	1-8-33	6	8

It is evident from the above experiments that the majority of adults do not survive for more than 3 to 8 days when the atmospheric humidity ranges from 30 per cent to 90 per cent at a constant temperature of 35°C. The influence of low humidity is detrimental to the life of the mosquitoes, since 75 per cent mortality occurred in 3 to 4 days when the relative humidity was 30 per cent.

The effect of lowering the temperature is favourable to the increase in longevity. At a temperature of 30°C. (86°F.), when kept in the desiccators running at 30 per cent, 50 per cent, 70 per cent, and 90 per cent relative humidity, the females survive for comparatively much longer times. Again, the effect of low humidity is evident, since at 30 per cent relative humidity the average life of the mosquitoes did not exceed 6 days. As we raise the humidity,

the duration of life is much enhanced. The results of these experiments have been summarized in Table II and also in Chart I.

CHART I.

Survival of *Anopheles subpictus* under varying conditions of temperature and humidity.

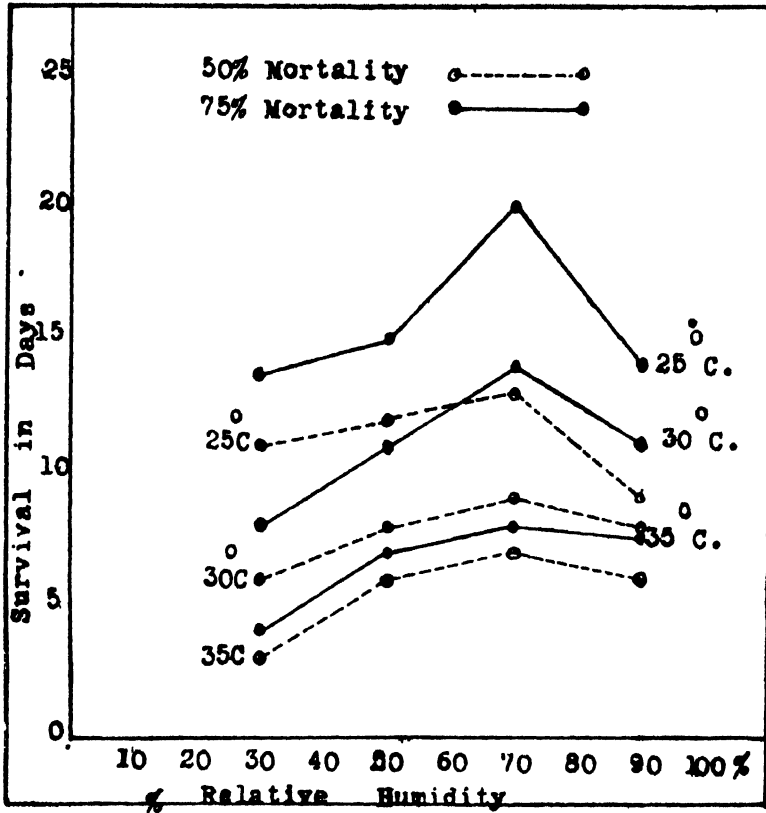


TABLE II.

Constant temperature 30°C. (86°F.).

Experiment number.	Number of females.	Relative humidity, per cent.	Date females emerged.	Date of blood meal.	50 per cent mortality in days.	75 per cent mortality in days.
1	27	30	9-7-33	10-7-33	6	8
2	40	50	8-7-33	9-7-33	8	11
3	40	70	8-7-33	9-7-33	9	14
4	40	90	8-7-33	9-7-33	9	11

A lowering of temperature to 25°C. (77°F.) further prolongs the duration of life. Out of a total of 32 females subjected to this temperature at 30 per cent relative humidity, 50 per cent of them lived for 11 days and 75 per cent mortality occurred in 14 days (Table III).

TABLE III.
Constant temperature 25°C. (77°F.).

Experiment number.	Number of females.	Relative humidity, per cent	Date females emerged	Date of blood meal.	50 per cent mortality in days	75 per cent mortality in days
1	32	30	23-8-33	24-8-33	11	14
2	32	50	2-9-33	3-9-33	12	15
3	36	70	30-8-33	31-8-33	13	20
4	32	90	30-8-33	31-8-33	9	14

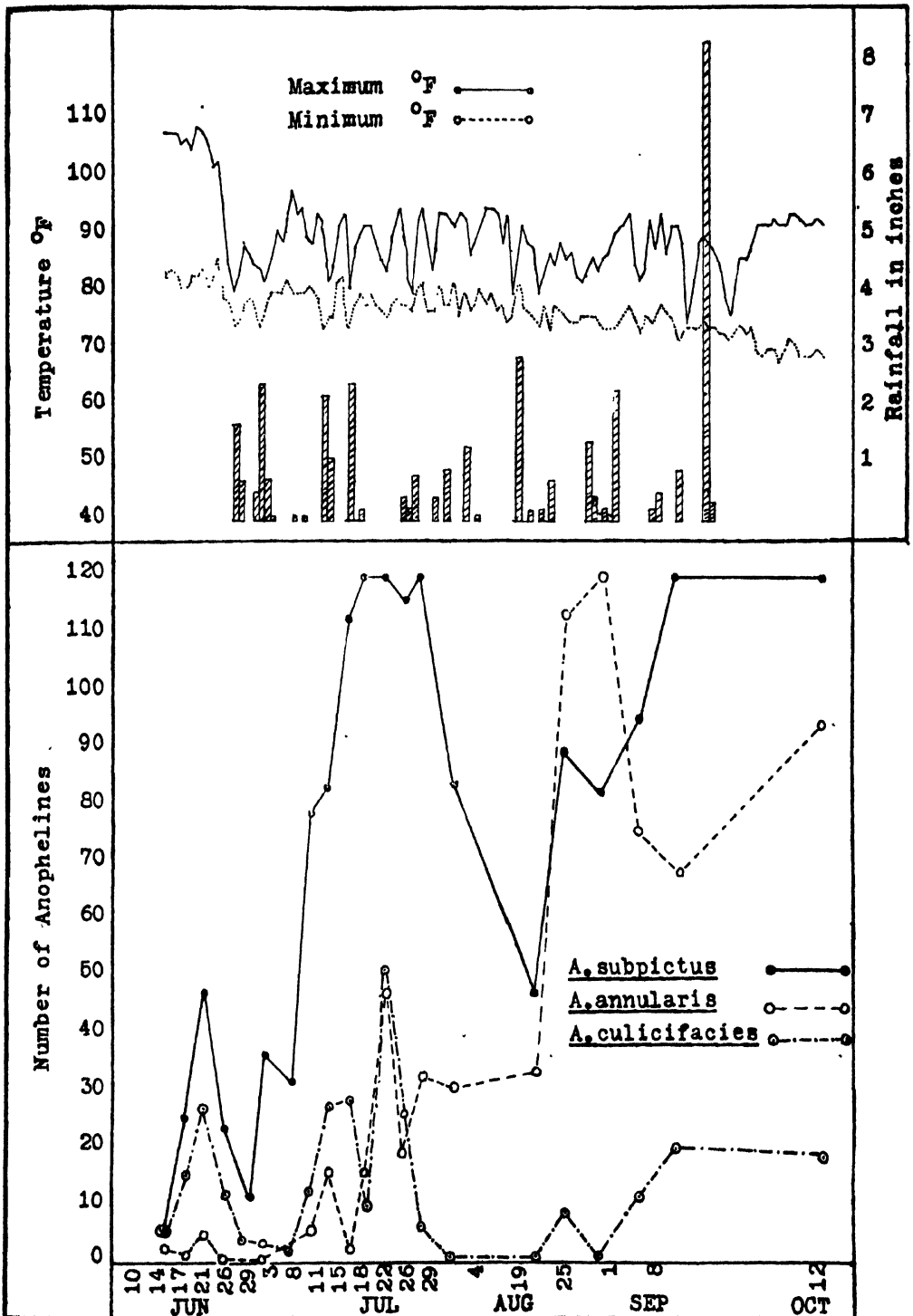
At higher humidities (50 per cent and 70 per cent), the average life of the imagines was recorded to be from 12 to 13 days. It is also observed that at a very high humidity, almost to the saturation point, the duration of life is very much curtailed. Out of a total of 32 female mosquitoes that were kept in this atmosphere, 16 died in 9 days while 75 per cent mortality took place in 14 days. It appears that high atmospheric humidity, about 90 per cent, retards the duration of life in this species. The most favourable degree of humidity in all the above experiments appears to be about 70 per cent, as it affords the maximum longevity at all temperatures studied (Chart I). The exact mechanism of the detrimental effect due to high humidity (90 per cent) is not known. It is quite likely that the physiological condition of these insects does not allow them to regulate their heat by evaporation.

INCIDENCE OF *ANOPHELES SUBPICTUS* DURING SUMMER AT KARNAL.

For comparison with the results obtained in the laboratory, a record was kept of the number of adults of *Anopheles subpictus* caught from the 'Catching Station'. For this purpose two rooms in Barrack No. 11, at the Imperial Cattle Breeding and Dairy Farm, Karnal, were selected, and catches were made from June to September 1933. This place is inhabited by human beings as well as by cattle. The results of adult catches have been incorporated in Chart II, which also includes observations on the other two species of *Anopheles*, viz., *A. annularis (fuliginosus)* and *A. culicifacies*.

It was observed that in June when the maximum temperature in the shade rose to 108°F., and the minimum recorded was about 85°F., the number of females of *Anopheles subpictus* collected in this area averaged only 22 per catch. Later, in July and August, with the advent of the monsoon rains, the number of insects began to rise and the average number of imagines collected

CHART II.
Seasonal incidence of anophelines at Karnal



rose to 104, as against an average of 22 in the catches made in the previous month. Similar collections were made in September, and the number of mosquitoes was approximately the same as had been observed during August. The daily maximum temperature is very much lowered at the latter season due to increased and heavy rainfall and the atmosphere also becomes more humid, thus presenting more favourable conditions for the survival of the females. The biotic potential of the insect is consequently raised and this explains the presence of these mosquitoes in conspicuously large numbers during August and September.

DISCUSSION.

As is true of all ecological factors, the temperature and humidity are not only associated in their continual fluctuations in nature, but also the nature of the effect of each is modified by changes in the other. For instance, in *Anopheles subpictus*, it has been shown above that at 35°C. (95°F.) the majority of females do not survive for more than 4 to 8 days, this fluctuation being essentially dependent upon the prevailing atmospheric humidity. Low humidity combined with this high temperature shortens the duration of life, because of the rapid rate of evaporation and consequent loss of water from the body of the insect, with which it cannot cope. This is abundantly clear in my experiments conducted at 35°C. (95°F.) and 30 per cent relative humidity. But if this loss of water be partially compensated by keeping the mosquitoes at higher humidities (50 per cent to 70 per cent), a gradual increase in longevity results. Again, when similar experiments are carried out at comparatively lower temperatures (30°C. and 25°C.), the detrimental effect of low humidity is easily demonstrated. In this respect my observations essentially confirm the findings of other workers (Senior-White, 1932, and Mayne, 1930).

In his experiments on the longevity of *Anopheles*, Mayne (1930) has observed under the conditions of his experiments that 'the three anopheline species, *A. culicifacies*, *A. subpictus*, and *A. fuliginosus* did not survive more than 24 hours at relative humidities 40 to 42 percentage at temperatures up to 87°F. At relative humidities of 46 percentage, 42 specimens of *A. culicifacies* died within 4 days and 228 specimens of *A. subpictus* died in 3 days'.

It is true that low humidity is injurious to the life of the mosquitoes, but the extraordinary nature of the results quoted above from Mayne (1930) cannot escape notice. It is not easily conceivable that the three species of anophelines studied by him could not survive for more than 24 hours in the laboratory at temperatures up to 87°F., and humidity varying from 40 to 42 percentage. It has been observed that, when the conditions of temperature and humidity recorded in nature are very similar to those used by Mayne (1930) in his laboratory experiments, both *Anopheles fuliginosus* (*annularis*) and *A. culicifacies* occur in moderately large numbers. Such an observation does not support the contention that these meteorological conditions lead to the death of these species in 24 hours. It may be argued that the recorded meteorological conditions are only a general condition, and that the insects seek micro-climates more suitable for their existence. This may be and probably is so, but the carefully conducted experimental evidence given in this paper has failed to confirm the very high mortality reported by Mayne (1930).

Relative humidity above 50 per cent is more favourable for the life of this mosquito, and the maximum longevity occurs at 70 per cent, taking into consideration all the temperatures studied.

It has also been found that humidity of 90 per cent is harmful for the survival of this mosquito. This is particularly marked at comparatively lower temperatures (25°C. and 30°C.) (Tables II and III). Similar conclusions have also been arrived at by Martini and Teubner (1933), who have observed that excessive humidity is harmful for the longevity of *Anopheles maculipennis*. Again, in some Diptera, Beattie (1928) has also been able to show that high humidity is detrimental to the life of those insects at lower temperatures because they are unable to control their heat by evaporation. Whether this explanation is correct or not, it is difficult to say, and certainly this aspect of the problem needs further investigation. It is, however, understood that the degree to which these mosquitoes are affected by a densely humid atmosphere depends on their individual physiological condition. Perhaps individual variations in this respect might explain why some anophelines are comparatively scarce during the rainy season in the Punjab, when the temperature is favourable for their survival.

From the foregoing account it is clear that the majority of the adults of *Anopheles subpictus* do not survive for more than 5 to 11 days, taking into consideration the variations in the atmospheric humidity and the temperature ranging about 30°C. (86°F.). Gill (1925) stated that this insect must be able to survive for at least 12 days after imbibing infected blood before it can play any part in the spread of malaria. Thus it is abundantly clear from the experimental evidence given above that the females of this mosquito will not play any part in the transmission of malaria, as they do not live long enough for the sporozoites to develop. We have some evidence (Mayne, 1930, and others) that *Anopheles subpictus* can be infected in the laboratory, and it has been possible to demonstrate the presence of viable sporozoites in the salivary glands of this mosquito. There is, therefore, nothing in the internal system of the insect to prevent the formation of oöcysts, or in any way cause their degeneration. But from the fact that in India we do not find any mosquitoes in nature even with gut infection, it seems that there is some other explanation to seek for the solution of this problem. That the adults cannot survive long enough for the sporozoites to develop offers a very plausible explanation. But to regard this as the only explanation does not appear possible, since we find that in the majority of cases the females survive about 5 to 11 days at 30°C. (86°F.) and consequently we should expect some oöcysts in the stomach of these mosquitoes, which is not in accord with the evidence available. Further work on these lines might elucidate the other factors involved in the solution of this problem.

SUMMARY AND CONCLUSIONS.

The method and technique for the control of humidity and temperature in relation to the study of the effect of physical factors on the longevity of some Indian anophelines have been described.

At 40°C. (104°F.) the females of *Anopheles subpictus* do not survive for more than 24 to 50 hours when the humidity ranges from 50 to 90 per cent. The duration of life is comparatively enhanced when the temperature is lowered to 35°C. (95°F.). It has been found that at this temperature, with

humidity varying from 30 to 90 per cent the mosquitoes live from 3 to 8 days. Low humidity is detrimental to the longevity of the females. At 30°C. (86°F.) the females live from 6 to 14 days and the maximum life has been recorded at 70 per cent relative humidity. At still lower temperature of 25°C. (77°F.) the adults live for a much longer time, extending to 20 days or even more in some cases.

High humidity, about 90 per cent, is injurious to the life of the females of this species, and this is particularly marked at 25°C. (77°F.).

It is concluded that the females of *Anopheles subpictus* live, for the most part, from 5 to 11 days at 30°C. (86°F.), and therefore the sporogonous cycle of the malarial parasite cannot be completed during the life of the mosquito host. This is one of the important factors elucidated to explain why *Anopheles rossii* (*subpictus*) is not a 'carrier' of malaria in nature.

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NOTE ON THE CONTROL OF MOSQUITOES AND MALARIA IN DELHI.

BY

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[18th May, 1934.]

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THIS note is the result of a visit paid to New Delhi on 14th and 15th April, 1934, at the request of the Public Health Commissioner with the Government of India. I was asked to advise on the following matters :—

- (1) The prevailing mosquito nuisance in New Delhi.
- (2) The control of malaria.

I. THE MOSQUITO NUISANCE IN NEW DELHI.

In order to understand this problem, it is necessary to know something about the seasonal prevalence of mosquitoes in this part of India.

During the early spring there is an enormous increase in culicine mosquitoes, commencing at the end of March, reaching its greatest height in April, and dying away in May. The rise in the prevalence of anopheline mosquitoes commences rather later in the year, beginning in April, reaching its greatest height during the first fortnight in May, and dying away in June. At the onset of the rains there is a second rise in the number of anophelines, to a much higher level than in the spring. The culicines do not participate in this second rise (*vide* Hodgson, 1914). These seasonal variations in the prevalence of mosquitoes are not peculiar to Delhi, but occur throughout the Punjab.

The mosquito nuisance which is complained of in Delhi at this time of the year is entirely due to the prevalence of culicine mosquitoes, chiefly *Culex fatigans*. This mosquito breeds in any kind of stagnant water, and will tolerate a high degree of organic pollution; indeed, it breeds most readily in foul water. Favourite breeding places are storm-water drains with their catch-pits, sullage pits, disused wells, water in disused tins, pots, barrels, garden tanks and receptacles of all kinds, sump-pits, quarry pits, water used in building construction, borrow-pits, etc. It will breed in the cisterns and pans of water-closets and in the traps of bath-rooms, whenever these are not in daily use. When suitable breeding places are present larvæ appear in enormous numbers, so that a single collection of water may produce many thousands of mosquitoes. It is thus easy to understand that unless the greatest care is taken, not only by the anti-mosquito staff, but also by the house-holders themselves, an annual recrudescence of the spring mosquito nuisance, in greater or less degree, is inevitable.

About the year 1922, when a large amount of building was going on in New Delhi, complaints of plagues of mosquitoes were common. Colonel S. R. Christophers, I.M.S., whose advice was sought found that the drainage system then being installed favoured the breeding of mosquitoes, through the provision of the usual sump-pits and other openings (Christophers, 1923). A design for a mosquito-proof system of drains suggested by him was adopted, and satisfactory traps were subsequently installed by the Chief Engineer and his staff under the supervision of Colonel Stanger-Leathes, I.M.S., Health Officer, New Delhi. Christophers (1927) recorded that 'at present all mosquito nuisance is absolutely in abeyance'.

For some years after this the spring prevalence of mosquitoes seems to have been kept within bounds, but in the spring of 1931 the Director, Malaria Survey of India, was asked to send an officer to investigate the mosquito nuisance, and to report on the probable effect of the Kilokri Sewage Farm on the incidence of malaria in New Delhi. The Sewage Farm lies due south-east of New Delhi, and is distant $1\frac{1}{2}$ miles from Prithvi Raj Road, 2 miles from Akbar Road, and $3\frac{1}{2}$ miles from Viceregal Estate. It covers 1,600 acres of land, 1,200 acres of which are allotted for irrigation. The Farm was opened on 20th February, 1926. Prior to this date the sullage of Delhi City was disposed of on land near Feroze Shah Kotla, whilst the sewage of New Delhi during the construction of the New City was disposed of on land situated to the north of the present disposal area. There was apparently no suggestion that the existence of the Farm was responsible for any increase in mosquitoes in New Delhi before 1931.

Dr. G. Macdonald, Malaria Research Officer, visited New Delhi on 19th and 20th May, by which time the plague of mosquitoes had ceased. The Sewage Farm had been cited as the source of the plague of mosquitoes on the grounds that the insects were most numerous in Prithvi Raj Road and Aurangzeb Road, which formed the south-eastern limits of inhabited New Delhi; that it had been impossible to find any breeding places nearer than the Farm, on which numerous breeding places were known to exist; and that there was frequently a steady wind blowing from the direction of the Farm towards Prithvi Raj Road, which would assist in carrying the mosquitoes.

In his report Macdonald (1931) noted that there had been a great rise in the subsoil water level at the Farm in the past 9 years. In 1925 it had been

30 to 40 feet below ground level; in 1926 it was between 20 and 35 feet; in 1928 it was within 20 feet of ground level over practically the whole area, and in places within 10 feet; whilst by March 1931 there had been a further rise of about 5 feet, so that the ground was in a dangerously water-logged condition.* This was aggravated by the practice of pumping back on to the Farm water which would otherwise flow off down a certain nullah.

Macdonald concluded that the recent plague of mosquitoes probably originated in the Sewage Farm, or from the untreated lower reaches of the Ganda Nullah, a south-easterly wind assisting them in their flight. His opinion was that although the Sewage Farm was not a direct menace to New Delhi as regards malaria, it was a serious cause of the disease in the surrounding villages, and thus might prove a danger to New Delhi should anti-larval measures ever fail within the New City, by providing a reservoir of infection. He recommended that measures should be taken to reduce the subsoil water level, and that the system of pumping back the water draining off into the nullah referred to above should cease. The latter recommendation was subsequently carried out.

Apparently, in 1932 and 1933, the spring mosquito nuisance was not excessive; but in 1934 the number of culicines was again large, and complaints of the mosquito nuisance were received from various quarters, including Viceregal Estate. This led to the present investigation, which took place on 14th and 15th April, 1934.

In the short time available, it was naturally impossible to make a detailed investigation. A visit was paid to the Sewage Farm, and culicine mosquitoes were found breeding in considerable numbers, in various drains and water collections.

I also visited a compound in Prithvi Raj Road, the occupant of which had complained that the mosquito nuisance in his bungalow was very bad. A large number of new buildings are being constructed in this vicinity. In the plot of land adjoining the bungalow in question were a brick sump and an iron receptacle left by the builders, both containing water in which were culicine mosquitoes in countless numbers. These two collections of water would be sufficient to account for any mosquito nuisance in the immediate vicinity, for they must have produced thousands of culicines daily. A cistern on the roof of one of the servants' latrines in the vicinity was inspected. No larvæ were found in it, but as its cover was merely a removable piece of wood, it constitutes a potential breeding place. The same remark applies to a large well in the compound.

Culicine larvæ in moderate numbers were also found in some borrow-pits near the railway embankment in the vicinity of Q point, and in certain other water collections remaining over from the excessive rainfall of the previous autumn in pits near the Muttra Road.

* The subsoil water level has continued to show a steady rise up to the present date. The average daily amount of sewage pumped on to the Farm in recent years is as follows:—

1928-29	5'1 million gallons.
1929-30	6'0 " "
1930-31	7'1 " "
1931-32	7'3 " "
1932-33	7'8 " "
1933-34	9'3 " "

CONCLUSIONS.

There is no doubt that the Kilokri Sewage Farm produces culicine mosquitoes in large numbers. But it seems to me highly improbable that it is the chief source of the mosquito nuisance in New Delhi, for the following reasons :—

(1) In the vast majority of cases where complaints are made of the mosquito nuisance in any locality, the source of breeding is found to be in the *immediate* vicinity. The instance cited above in respect of the bungalow in Prithvi Raj Road is a case in point. Another instance, which is even more striking, in that it illustrates the difficulty which may be experienced in locating such breeding places, occurred in New Delhi, in August 1930. Mosquitoes were numerous in the bungalow occupied by Major J. R. D. Webb, I.M.S., then Health Officer, New Delhi, in spite of a vigorous anti-mosquito campaign. He and Dr. Macdonald, who was then visiting Delhi, spent a considerable time searching for the source of these mosquitoes, but without success. Subsequently three previously undetected breeding places were discovered. One was a large earthenware pot, which had been suspended from a tree to provide drinking water for birds. The others were two sump-pits and some old tins in an unoccupied house nearby. All these breeding places were literally black with mosquito larvæ.

Delhi also provides a striking instance indicating that a locality may be very little affected by mosquitoes, even though they are being produced in large numbers, provided that the breeding places in the immediate vicinity are efficiently controlled. In spite of the most profuse breeding in the fruit garden area, the Roshanara Club was freed from the mosquito nuisance by vigorous control in Roshanara Gardens and its immediate neighbourhood. As the result of his work in New Delhi, it was Major Webb's considered opinion that if the immediate vicinity was completely freed from all possible breeding places, those outside the half-mile radius could be safely ignored.

(2) The fact that I found profuse breeding in the plot adjoining a bungalow in Prithvi Raj Road, the occupant of which had complained of the mosquito nuisance some days before my visit. If such a breeding place can exist untreated after complaints have been made, it is exceedingly probable that there are other undetected breeding places, especially among the large number of new buildings now in the course of construction.

(3) In the years 1932 and 1933, I understand that there was no excessive prevalence of mosquitoes in New Delhi, although the Sewage Farm was already water-logged. Anti-mosquito work was carried out in certain sections of the farm in the spring of 1931 and 1932, but not in 1933.

(4) The mosquito nuisance is said to be worse when the wind blows steadily from the Farm towards Delhi. Records supplied from the Meteorological Office, New Delhi, taken at 8-21 a.m. and 5 p.m. during the period 15th March to 15th April, 1934, show that on only one occasion (9th April at 8-21 a.m.) was the wind blowing from the direction of the Farm (S. S. E.) at those hours. The prevailing wind during this period was W. N. W. (42 records), i.e., directly from New Delhi to the Farm. On three occasions it was W. S. W., and on one occasion E. N. E. whilst on 8 occasions conditions were recorded as 'calm' (*vide* Appendix II).

(5) A record of trap-catches of mosquitoes taken in the Prithvi Raj Road area on 19th April, 1934, kindly supplied by the Health Officer, New Delhi, gave the following figures :—Females 24, males 20 (all culicines). The large proportion of males captured indicates that the source of breeding was in the immediate vicinity.

(6) The relative humidity of the atmosphere is very low during this period. Long flights of mosquitoes seldom, if ever, occur unless the relative humidity is very high, *e.g.*, just before the onset of the rains. Further, with low relative humidity, the life of mosquitoes is very short, so that in order to keep up the numbers the flights would have to be made at very frequent intervals.

For these reasons I think that although the Sewage Farm may play a part in the production of the spring mosquito nuisance in New Delhi, it is highly probable that most of the trouble is caused by local breeding in New Delhi itself. The question as to the actual part played by the Sewage Farm could only be settled by observations at selected catching stations situated at many different points throughout the area. It would be necessary to record the numbers caught at each station during a stated period, and the relative proportion of males to females captured. Such an experiment, if carried out scientifically, would probably be of considerable value. In judging the result, it would of course be necessary to take into consideration any factors which might affect local conditions, for example, the extensive building operations now in progress in various parts of New Delhi.*

RECOMMENDATIONS.

(1) If I am correct in attributing the greater part of the spring mosquito nuisance to localised breeding, it follows that the anti-mosquito organisation has failed in its object. Supposing that funds are available for supplying sufficient oil and appliances, the implication is either that the staff are insufficient for their task, or that they are not doing their work thoroughly. The latter usually implies lack of adequate supervision.

The area of New Delhi is approximately 32 square miles, and there are in it 784 bungalows. There is an anti-mosquito staff† consisting of one mate, 14 beldars, 19 sweepers and 2 pipe fitters. The Sanitary Superintendent is in charge of the whole area, which is divided into 8 sections. The work is supervised by 8 jemadars in charge of sections, 4 daroghas in charge of wards, 2 inspectors in charge of circles, and one naib daregha in charge of central waterways and outfalls. Two men are allotted to each section, and the remainder

* Buildings are under construction at present in the following localities:—

Block 17 near Tughlak and Safdarjung Road.
 Blocks 18 and 12 near Tughlak and Aurangzeb Road.
 Block 13, Albuquerque Road.
 Blocks 14 and 15, between points E and Q.
 Blocks 200 and 201, between Ferozeshah Road and Canning Road.
 At point R/5 and Block 90, near Lady Harding Road.
 Block 81, near Punchkuin and Kutab Road.
 Railway area between Kutab Road, and the road for State Entry.
 Areas adjoining Minto Road and Circular Road.
 Block east of the Press Quarters.
 South of the plot reserved for the Irwin Hospital.

† The staff and budget allotment of anti-mosquito work in New Delhi, the Notified Area, Delhi City, and Viceregal Estate are given in Appendix I.

are engaged on the major breeding grounds, such as Q point outfall, large pits near the Muttra Road, quarries on the Ridge and brickfield area, etc. The sections are visited at least three times a week.

The budget allotment for anti-mosquito work in New Delhi is Rs. 15,000, of which Rs. 8,320 is taken up by salaries of the staff.

It is impossible for me to say whether this staff is sufficient to deal with all the breeding places over such a large area. This can only be judged by the officers under whom they work. Presumably the staff has been found sufficient in the past, but it seems possible that the recently renewed activity in building operations and the extension of the inhabited area may have so increased the work that it cannot adequately be carried out without some addition to the staff.

However this may be, the most important point is adequate supervision. I do not think this will ever be satisfactorily carried out until a special officer is appointed, who has no duties whatsoever beyond the control of malaria and mosquitoes. This question is dealt with in the second part of this note.

(2) I understand that a scheme for the removal of the Sewage Farm to a more distant site, coupled with an improved method of sewage disposal, is under the consideration of Government and likely to be adopted. The removal of the Farm is most desirable on general sanitary grounds, and also from the viewpoint of malaria control, for the water-logging of the soil, by preventing the monsoon rainfall from soaking in, favours the incidence of malaria, more especially in the neighbouring villages.

It seems highly probable, however, that the Farm will still be in its present location next spring. I would suggest that an estimate be prepared for clearing and oiling the drains in which most of the breeding is found to occur during the six weeks commencing from 15th March. It must then be decided whether the expenditure of this money for the mitigation of what after all is only a temporary discomfort is justified, or whether such funds as are available should be devoted to the actual prevention of disease.

(3) I think much good could be done by the issue of a circular to all occupiers of houses in New Delhi, explaining that a plague of mosquitoes is inevitable in March and April unless they co-operate in the anti-mosquito campaign. The chief breeding places should be cited, and it should be explained that these should be treated with oil, cresol or phenyl once a week. Residents should also be advised to see that any case of fever occurring in their compounds receives adequate treatment, since servants and their families, especially children, are often an important source of malarial infection.

(4) I am informed by the Health Officer, New Delhi, that the Municipality has no powers to prosecute occupiers of houses for permitting mosquitoes to breed on their premises. Until such legal powers are conferred it is unlikely that the mosquito nuisance will be adequately dealt with.

(5) All wells* should be either filled in or provided with a cement concrete cover, with a pump if necessary. No trap doors should be allowed. Detailed

* There are 165 wells in the New Delhi area, of which 113 are situated more than half a mile from the periphery of the inhabited area, and are disused. Of the remainder 33 are unprotected and 19 are covered, but only six of these are mosquito-proof.

instructions for dealing with wells are laid down in 'Malaria in Bombay, 1928' (Covell, 1928). Disused wells are a prolific source of culicines, and until they can be dealt with by permanent measures they should be regularly oiled.

(6) All cisterns* should be mosquito-proof. Detailed instructions on all the essential points regarding the mosquito-proofing of cisterns are laid down in 'Malaria in Bombay, 1928', and in 'Malaria in Calcutta' (Covell, 1932). In the latter publication the principal points are illustrated in a diagram.

II. THE CONTROL OF MALARIA IN DELHI.

Before discussing this question it will be useful to give a brief account of the numerous expert investigations which have been made with regard to malaria in Delhi, the recommendations which have been put forward, and the action which has been taken to carry them out.

The earliest report available is that of the Commission of 1845, which showed that the water-logging caused by the Western Jumna Canal was responsible for the malarious conditions along its course (Baker, Dempster and Yule, 1847).

Apart from this, investigations made prior to 1912 dealt chiefly with Delhi Fort and Daryaganj Cantonment, which were notorious in the Army as hotbeds of malaria. Reports dealing with malaria in these localities were made by Lelean (1909), Adie (1911) and Hehir (1911).

In 1914 Hodgson published his report, the result of 16 months' exhaustive investigations carried out in 1912-13, in connection with the proposed site of the New City. His chief recommendations are summarised below :—

(1) *Western Jumna Canal area*.—Canal to be cut off entirely and filled up. Irrigation to be allowed from wells. Drainage of railway borrow-pits and surface drainage.

(2) *Daibar area*.—Precautions against flooding. Canalisation of creeks and nullahs.

(3) *City*.—Treatment of Bela. Closing of canal to Queen's Gardens, etc. Abandonment of Daryaganj Cantonment. City not to be extended in this direction. Wells to be filled in or hermetically sealed.

(4) *New City area*.—Properly built storm-water channels. Filling up of brickfields, wells and pits of all kinds. Precautions against collections of water in quarries. Okhla Navigation Cut to be filled up, and removal of all bunds and obstruction to drainage. Proper drainage of all railway borrow-pits. A strip of land one mile wide to be left clear of all buildings alongside of the Bela and converted into a park. No canals to be brought into this area. Gardens to be watered from piped supply.

Action taken.

(1) Masonry canalisation of a large number of drains north of the city, including Khudsia Creek, and parts of the Najafgarh Cut and Metcalfe House Nullah.

* The number of cisterns in the New Delhi area is 966.

(2) A system of stone-pitched drains was constructed parallel with the Jumna along the Upper Bela Road.

(3) The Western Jumna Canal at the corner of the City by the Lahori Gate was closed, and the bed of this south of Paharganj was filled in.

(4) All branches of the canal within the city walls, except one for the irrigation of Queen's Gardens were closed or converted into sewers.

(5) A policy of filling in depressions in the Bela with City rubbish was inaugurated.

(6) The greater part of the Okhla Navigation Cut was filled in.

The next investigation was that of Colonel Christophers in 1922, referred to in the first part of this note, and this was chiefly concerned with the mosquito nuisance in New Delhi. His report (1923) was entitled 'Note on the prevention of the introduction of malaria and of the mosquito nuisance into the New Imperial City of Delhi'.

In 1926, following a severe outbreak of malaria, the advice of Colonel Christophers was again sought. He pointed out the following defects in the action taken on Hodgson's report (Christophers, 1927) :—

(1) Failure to have excluded canal irrigation from the residential area north of the city.

(2) Failure to have completely obliterated and filled in the cul-de-sac of the Western Jumna Canal where this lies close up against the Sadar Bazar and Lahori Gate.

(3) Failure to have filled up the empty bed of the canal between the Lahori Gate and Paharganj.

(4) Failure to have dealt with the Bela where it lies over against the Fort and the City.

To this list may be added :—

(5) Failure to close or hermetically seal the wells. New wells were actually allowed to be dug in New Delhi.

(6) Failure to deal with the borrow-pits, or prevent the digging of new ones.

Christophers' chief recommendations were :—

(1) That a new complete malaria survey be made of the Delhi area.

(2) That a Malaria Engineer should be appointed to carry out the recommendations made. The survey was not recommended unless the Government were prepared to associate with it a Malaria Engineer.

(3) That a Committee be formed to give the proposals the required backing, of such a strength that its word should be practically final.

Action taken.

A malaria survey was carried out by Mr. Senior White, Research Officer, Malaria Survey of India, during 1927 and the early months of 1928. His

report (Senior White, 1930), submitted in 1928, contained the following recommendations :—

- (1) Western Jumna Canal to be closed from Pembari Bridge and filled in to its tail at Pul Mithai.
- (2) Stone pitching of margin of Roshanara Tank.
- (3) Najafgarh Cut to be stone-lined with cunette from junction of Dahalia Escape to Kalasi Nehar, and from Alipur Road to its termination.
- (4) A new system of drains to be installed from near the end of Najafgarh Cut parallel with the Jumna, to take the flow in the Cut, Khudsia Creek, Fort Pukka Nullah and Metcalfe House Nullah southward to Q point Nullah. Subsidiary pumping stations to be provided where necessary. Outfall to be beyond the New City at Kilokri.
- (5) All bridge and culvert openings in the Agra-Delhi Chord to be closed from Hardinge Avenue Bridge to beyond Nizam-ud-Din.
- (6) Bela to be dusted with paris green from air-craft.
- (7) Filling in of all borrow-pits, and prohibition of further excavations. Drainage of Ridge quarries.
- (8) Irrigation on Bela to cease.
- (9) Closure of all wells.
- (10) Legal tightening and much stricter application of the existing ordinances affecting mosquito breeding.
- (11) Appointment of a Malaria Officer with executive powers on the staff of the A. D. P. H., Delhi Province.

Notes on this report were written by the Public Health Commissioner and the Director, Malaria Survey of India.

On 28th May, 1928, at the instance of the Director-General, I.M.S., a meeting of the Delhi Anti-malarial Committee* was held in Simla, to consider the proposals for anti-malarial work in Delhi, as put forward in the reports of Christophers and Senior White, and noted on by the Public Health Commissioner and the Director, Malaria Survey of India. The following is an abstract of the resolutions passed :—

- (1) The urgent necessity of making a serious effort to create a malaria-free enclave for the Imperial City is stressed.
- (2) The Western Jumna Canal should be stopped at Pembari Bridge and filled in as suggested by Senior White, irrigation being permitted from closed shallow wells.
- (3) Senior White's proposals for Roshanara Tank are approved. Roshanara Gardens to be supplied by closed wells, as above.
- (4) Najafgarh Cut should be stone-lined as suggested by Senior White.

* This Committee was composed of as follows:—

Director-General, I.M.S.	} President.
Mr. A. M. Rouse, C.I.E., P.W.D.	
Lieut.-Col. F. P. Mackie, V.H.S., I.M.S.	
Lieut.-Col. H. Stanger-Leathes, I.M.S.	
Major J. A. Sinton, V.C., I.M.S.	
Lieut.-Col. A. C. Munro, I.M.S.	} Members.

(5) No action should be taken on Senior White's proposal No. 4, until the results of the work suggested in the present Resolutions Nos. 2, 3, 4 and 6 are seen.

(6) The possibility of reducing flooding and causing a permanent fall in the subsoil water level by permitting a freer exit of water through the Okhla headworks, during the monsoon period at least, requires immediate investigation in conjunction with the canal authorities as being of vital importance to the area south of Delhi, which includes the New City.

(7) One-way sluices should be inserted in the bridge and culvert openings in the embankment of the Agra-Delhi Chord instead of Senior White's recommendation No. 5.

(8) The R. A. F. should be approached as to the feasibility of using paris green for dusting the Bela.

(9) It is suggested that a *pukka* embankment rising one foot above normal high water level should be built, extending from the Electric Power Station on the south to the railway bridge on the north. The Bela between this and the Fort should be filled up to a level of one foot above high water level.

(10) All borrow-pits should be filled in or drained. Further excavations of this nature should be absolutely forbidden. Brickfields should be levelled as far as possible. Quarries should be similarly dealt with.

(11) Cultivation on the Bela in the vicinity of the New City, *i.e.*, between the Power House and Purana Kila, should be prohibited and all the wells in this area should be filled in.

(12) All wells in New Delhi should be closed and the digging of new ones strictly forbidden. Steps should be taken at the earliest possible date to have all wells in the City filled in or otherwise permanently closed.

(13) A set of legal enactments should be drawn up to deal with mosquito breeding on private premises.

(14) It is essential that the A. D. P. H., Delhi Province, should have a whole-time Malaria Officer attached to his staff, and that this officer should have executive powers.

(15) The Committee agree with Colonel Christophers that the services of a Malaria Engineer are essential.

Action taken.

As far as I have been able to ascertain, of all the recommendations made by Senior White and the Delhi Anti-malarial Committee, the only one on which any action whatever has been taken is that referring to excavations. Certain borrow-pits have been filled in or drained on the Ridge, and a few disused quarries have been filled with refuse. On the other hand fresh excavations have been dug in other places, and there are probably at the present time more borrow-pits capable of breeding malaria-carrying mosquitoes in New Delhi than there were in 1928. This point will be referred to later.

During the years 1929 and 1930 Lieut.-Colonel Sinton, I.M.S., Director, Malaria Survey of India, visited Delhi on four occasions, and Dr. Macdonald,

Research Officer, Malaria Survey, on two occasions, to advise on matters connected with malaria. Colonel Sinton submitted three reports in connection with the proposed sites for the quarters of railway clearing house clerks, and one report on the subject of mosquito breeding in the Moghul Gardens. Dr. Macdonald submitted a detailed report on his visit to Delhi in August 1929. The report was chiefly concerned with temporary measures to be applied to the Western Jumna Canal, fruit gardens area, Najafgarh Cut and other channels, the Bela, ornamental waters and storm-water drains in New Delhi, wells, borrow-pits, etc., until such time as permanent measures could be carried out. Temporary measures, such as the application of oil and paris green to all breeding places in the area and for a half-mile radius outside it, have been carried out up to the present time by the anti-mosquito staff.

The last visit of Dr. Macdonald, which was made in connection with the spring mosquito nuisance in 1931, and the report submitted by him regarding the possible influence of the Sewage Farm on mosquitoes and malaria in New Delhi have been discussed in Part I of this note.

As is clear from the above brief summary, expert advice on the control of malaria in Delhi has been sought and given on many occasions during the last 25 years. Where it has been followed, as in the case of some of Hodgson's recommendations, the results have been excellent. Unfortunately only a very small proportion of the permanent measures recommended have been carried out, presumably owing to lack of funds. For the same reason the appointment of a whole-time Malaria Officer has not been sanctioned. Under the circumstances the only possible course has been pursued, *i.e.*, an attempt has been made, with varying success, to control mosquito breeding by means of temporary measures, as far as the funds available will permit.

RECOMMENDATIONS

As noted above, the recommendations of Christophers and Senior White, the latter based on a detailed survey carried out in 1927-28, together with the notes written on them by the Public Health Commissioner and the Director, Malaria Survey of India, were carefully considered by the Delhi Anti-malarial Committee in 1928. The resulting resolutions, which modified Senior White's proposals in certain respects, should form the basis of anti-malarial work in Delhi. It is certain that if they were thoroughly carried out the incidence of malaria would be very greatly decreased. These resolutions will not be recapitulated here, but the following comments seem called for :—

(1) Every one of the many experts who have been called in to investigate malarial conditions in Delhi has laid down that borrow-pits should be filled or drained and that in future all excavations of this nature should be strictly prohibited. Yet, during the last three years, fresh borrow-pits have been created as follows :—Excavations were dug in the vicinity of the Delhi-Muttra Road in Block 159. This matter was reported to the P. W. D., and action is now being taken to fill them in. Borrow-pits have been excavated north and south of the Lodhi Road for road and railway embankments, no attempt being made to take the earth from the numerous hillocks available. This matter has also been reported to the P. W. D., but so far without result. Extensive excavations have also been made for the embankment of the road approaching

the Power House and Pumping Station east of Delhi Gate. In places these excavations have been carried down to subsoil water level, and remain full of water during the greater part of the year, necessitating constant watching and the periodical application of larvicides.

It is difficult to refrain from the comment that a community which allows this state of things to continue after so many warnings deserves all the malaria and mosquito nuisance that it gets.

(2) Similarly, every investigator, from Hodgson onwards, has stressed the importance of wells as a major source of malaria in Delhi, and has recommended their closure. Yet, in 1927, fourteen years after Hodgson's survey, new wells were actually being dug in New Delhi.* Comment is superfluous.

(3) I understand that the prospect of the appointment of a whole-time Malaria Officer is exceedingly remote, on financial grounds. But the very fact that funds will not allow the adoption of permanent measures makes the appointment of such an officer more urgent. Constant supervision is necessary for the successful prosecution of temporary measures. The task of the officer in charge of anti-mosquito work is both difficult and arduous, more especially because he is required, at any rate in New Delhi, not only to control malaria, but also to deal with mosquito breeding in general. My own view is that the appointment of a Malaria Officer would be an economy, and that the question at issue is not 'Can Delhi afford a Malaria Officer?', but 'Can Delhi afford to be without one?' A Malaria Officer requires special training, and should the appointment be sanctioned it is suggested that the officer should attend the annual Malaria Course held at Karnal by the Malaria Survey of India before entering on his duties.

To summarise :—

(1) The Resolutions of the Delhi Anti-malarial Committee held in 1928 should be carried out in their entirety.

(2) Since it seems probable that lack of funds will cause delay in carrying out major works, every effort should at present be concentrated on controlling mosquito breeding in the Old and New Cities themselves and for a half-mile radius beyond their boundaries. It should at least be possible to deal with:—

- (a) Wells.
- (b) Cisterns.
- (c) Borrow-pits and excavations of all kinds.
- (d) Domestic breeding places.
- (e) Drains and Nullahs.
- (f) Ornamental waters.†

Above all, drastic action should be taken in the event of new breeding places being created in the future, and adequate legal powers should be provided to deal with mosquito breeding on private premises.‡

* 'I am aware of several new pukka wells which have of late appeared in the Prithvi Raj Road area' (Senior White, 1930, p. 317).

† See Lieut.-Colonel Sinton's note of 1934, 'Suggestions regarding the use of *Gambusia* in ornamental tanks at New Delhi'. In the course of my visit I suggested to the Health Officer, New Delhi that he should send his Superintendent to the Field Experimental Station of the Malaria Survey at Karnal, to see the conditions under which these fish are kept, and to bring back with him a further supply for use in Delhi.

‡ The relevant sections of the City of Bombay Municipal Act might well serve as a model for Delhi, vide 'Malaria in Bombay, 1928', Chapter VIII, 'Anti-malarial Legislation'.

(3) As regards the major works recommended, in the carrying out of which lies the only hope of a permanent improvement in the situation, estimates should be prepared, so that they may be put into operation without delay when funds become available.

(4) It is not considered that the control of mosquitoes and malaria in Delhi will ever be really satisfactory until a whole-time fully-trained Malaria Officer is appointed.

I wish to thank Major C. M. Ganapathy, M.C., I.M.S., Health Officer, New Delhi, and A. D. P. H., Delhi Province, for kindly supplying much of the information on which this note is based.

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APPENDIX I.*Anti-malarial staff and budget allotments for New Delhi, Notified Area, Delhi City and Viceregal Estate.***(a) NEW DELHI.**

1 Naib Darogha	at Rs. 32	plus Rs. 6 per mensem	..	Rs.	456
2 Mistries	" "	52 per mensem each	..	"	1,248
1 Mate	" "	20 " "	..	"	240
14 Beldars	" "	15 " " each	..	"	2,520
19 Sweepers	" "	12 " "	..	"	2,736
2 Mates for 4 months	" "	20 " "	..	"	160
16 Beldars „ 4	" "	15 " "	..	"	960
Total					Rs. 8,320
TOTAL BUDGET ALLOTMENT					Rs. 15,020

(b) NOTIFIED AREA.

Months.	Weeding and Edging.	Oiling.
April 1933	1 Mate 4 Beldars	1 Mate. 14 Beldars. 1 Rubbish Cartman.
May „	1 Mate 4 Beldars	7 Beldars. 1 Rubbish Cartman.
June „	2 Mates 8 Beldars	10 Beldars. 1 Rubbish Cartman.
July „	2 Mates 16 Beldars	15 Beldars.
August „	2 Mates 16 Beldars	15 Beldars.
September „	2 Mates 13 Beldars	17 Beldars.
October „	1 Mate	16 Beldars.
November „	5 Beldars.
December „	2 Beldars.
January 1934	2 Beldars.
February „	8 Beldars	2 Beldars.
		Total salaries Rs. 2,961-6-6
		TOTAL BUDGET ALLOTMENT .. Rs. 7,500

(c) DELHI CITY.

1 Inspector at	Rs. 25 for 6 months	Rs.	150
30 Sweepers „	„ 13 „ 6 „	„	2,340
Total					Rs. 2,490
TOTAL BUDGET ALLOTMENT					Rs. 4,000

(d) VICEREGAL ESTATE.

1 Fitter at	Rs. 40	plus Rs. 10 Cycle Allowance per mensem	Rs. 600
10 Sweepers at	„ 15	per mensem each „ 1,800
8 A. M. Beldars at	„ 15	„ „ „ „ 1,440
Total			.. Rs. 3,840
TOTAL BUDGET ALLOTMENT			.. Rs. 4,999

APPENDIX II.*Direction of winds in New Delhi from 15th March to 15th April, 1934.*

Date.			8-21 a.m.	5 p.m.
15th	March	..	W. N. W.	W. N. W.
16th	„	..	W. S. W.	W. N. W.
17th	„	..	W. S. W.	W. N. W.
18th	„	..	W. N. W.	W. N. W.
19th	„	..	W. S. W.	W. N. W.
20th	„	..	W. S. W.	W. N. W.
21st	„	..	W. N. W.	W. N. W.
22nd	„	..	W. N. W.	W. N. W.
23rd	„	..	W. N. W.	W. N. W.
24th	„	..	Calm.	Calm.
25th	„	..	W. N. W.	W. N. W.
26th	„	..	Calm.	W. N. W.
27th	„	..	W. N. W.	W. N. W.
28th	„	..	W. N. W.	W. N. W.
29th	„	..	W. N. W.	W. N. W.
30th	„	..	Calm.	W. N. W.
31st	„	..	W. S. W.	W. N. W.
1st	April	..	W. N. W.	W. N. W.
2nd	„	..	W. N. W.	W. N. W.
3rd	„	..	W. S. W.	W. N. W.
4th	„	..	W. S. W.	W. N. W.
5th	„	..	W. N. W.	W. N. W.
6th	„	..	W. S. W.	W. N. W.
7th	„	..	W. S. W.	Calm.
8th	„	..	Calm.	W. N. W.
9th	„	..	S. S. E.	Calm.
10th	„	..	W. S. W.	W. N. W.
11th	„	..	W. S. W.	W. N. W.
12th	„	..	W. N. W.	W. N. W.
13th	„	..	W. N. W.	Calm.
14th	„	..	W. N. W.	W. N. W.
15th	„	..	W. N. W.	W. N. W.

APPENDIX III.*Note on the coolie camps in New Delhi.*

A large amount of coolie labour is employed in the construction of buildings in New Delhi. There are two principal coolie camps, one near the Jail, and one near Prithvi Raj Road. The number of persons in these two camps, including families, is about 8,000. I understand that these people have been in New Delhi for many years, and that the majority of the children in the camps have been born there. A spleen examination of these children, taken in the same month each year, would afford valuable information as to the amount of malaria in the locality. If there is any considerable inward and outward movement among these people, it is important that they should come under observation periodically, in case there should be a large influx at any time from a malarious area. Adequate treatment for cases of malaria should be provided, otherwise they might form an important focus of infection. Care should also be taken to see that these people do not store their water in receptacles for days at a time, and thus provide a source of mosquito breeding.

MOSQUITO PREVALENCE AND MOSQUITO-BORNE DISEASES IN CALCUTTA CITY.*

BY

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[2nd July, 1934.]

INTRODUCTION.

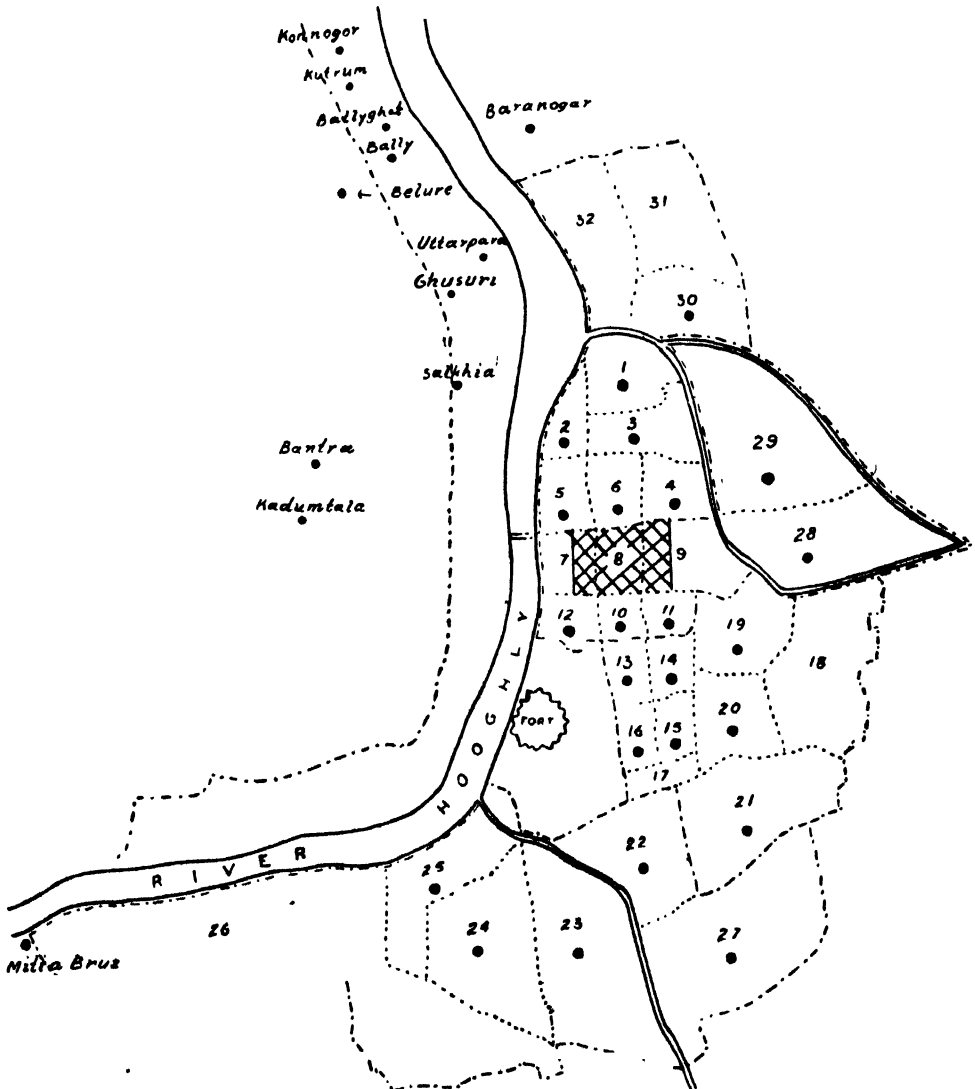
DURING the period of four years, July 1928 to June 1932, the breeding of *Anopheles stephensi* and of *Aedes aegypti* has been closely studied in an area one square mile in extent around the Calcutta School of Tropical Medicine. Observations on the breeding of *Culex fatigans* have also been carried out in the same area during the last two years of this period.

* *Note 1.*—The work recorded in this paper was carried out by the junior author, under a grant from the Indian Research Fund Association. The paper has been written jointly by both authors.

Note 2.—The protocols to this paper are too extensive to publish. They include as analysis hour by hour for each of the twelve months of the wet bulb readings, dry bulb, relative and absolute humidities, rainfall, maximum and minimum temperatures, and wind direction over a period of twenty years observed at the Alipore Observatory. They also show the prevalence month by month of the three species of mosquitoes concerned, details of the breeding places as observed month by month, of human immigration into Calcutta from different districts in Bengal with the malaria rates for those districts, of admissions for malaria, dengue and filariasis into the different Calcutta hospitals over a period of four years, and of gametocyte carriers month by month as observed in the out-patient department of the Calcutta School of Tropical Medicine over a period of four years. Two copies of these records have been preserved, and information with regard to them can be obtained either from the Director of the Calcutta School of Tropical Medicine or from the Director of the Malaria Survey of India.

The area studied consists of ward 8 and parts of wards 7 and 9, and is shown in cross hatching in Chart I. It is bounded on the north by Mechua-bazar Street and Cotton Street; on the south by Bowbazar Street, Lalbazar Street, and Dalhousie Square North; on the east by Amherst Street; and on the west by Charnock Place and Clive Street. In addition to this the limits of distribution of *A. stephensi* in and around Calcutta and Howrah were observed. This species was found breeding profusely throughout Calcutta city and Howrah, and as far north as Konnagar, nine miles north of Howrah railway station. The places in which *A. stephensi* was found breeding are indicated in Chart I by black spots.

CHART I.

Map of Calcutta and environs, showing breeding places of *A. stephensi*.

The following brief summary with regard to the meteorology and climate of Calcutta may here be given.

Calcutta is situated on the left bank of the Hooghly river, 86 miles from the sea. The soil is of recent alluvial deposits of the Gangetic delta, only 18 to 21 feet above mean sea level. The municipal area covers 31 square miles, and (including the Fort, Maidan, the Port and canals) has a population of 1,196,668. The modern city was founded by Job Charnock in 1690.

The climate is hot and moist. The average dry bulb temperature falls as low as 64.4°F. in December, and rises as high as 85.8°F. in May. The average maximal and minimal temperatures are 86.6°F. and 70.5°F. respectively. The average wet bulb temperature comes down to 58.6°F. in December, and rises as high as 80.1°F. in June. The mean relative humidity is 68 per cent in March and rises to 86 per cent in August and September. The mean absolute humidity is 0.422 in December and 0.974 in July. The average annual rainfall is 60 inches.*

Calcutta has a filtered and an unfiltered water supply and underground drainage.

Previous work.—In former times Calcutta was very malarious. O'Malley (1914) quotes Wilson, Hamilton and Hunter as testifying to the ravages of fever at the close of the 17th century. 'Within a decade after Charnock finally landed on the deserted river bank in 1690 it had become a busy mart with 1,200 English inhabitants, of whom 460 were buried between the months of August and January in one year. The miseries of the fever-stricken band throughout 1690 and 1691 are not to be told in words'. 'The early records of the East India Company prove that Calcutta and Bengal were malarious from the very commencement of the British occupation' (Fry, 1912). In the autumn of 1756 British troops on board ship at Falta suffered very severely from fever. 'The exposure during the rainy season, coupled with bad food and other privations, brought on a malignant fever, which infected all the ships, and ultimately carried off a majority of the party, leaving the remainder in a wretchedly reduced and pitiable condition' (Orme, quoted by O'Malley, 1914).

The filtered water supply was first introduced into the city in 1870 and the unfiltered water supply in 1878, and, partly on account of this and with modern drainage and sanitation, malaria commenced to decline. 'In 1880 the Government of Bengal passed a resolution to the effect that the reclamation of the Saltwater Lake is a project which "the growing prevalence of fever in Calcutta" makes it desirable to see again brought forward' (Iyengar, 1931). Ross (1923) recorded that there was but little human malaria in Calcutta in 1898. In February 1900 Rogers (1900) studied the relationship of the drinking water, water-logging, and distribution of anopheline mosquitoes to the prevalence of malaria north of Calcutta along the bank of the Hooghly. In Chitpur-Cossipore he recorded the spleen rate as 11.2 per cent, the smallest figure obtained for any of the municipalities examined, a finding which he explained by the fact that this municipality had a filtered water supply, though water-logging was most abundant in this area. In the same year, 1900,

* These figures are derived from the averages of hourly records over a period of twenty years at the Alipore Observatory and may be taken as standard values.

Dr. Neild Cook, Health Officer of Calcutta, investigated the connection between the anopheline mosquitoes and malaria in the city of Calcutta. He found 89 tanks with abundant *Anopheles* larvæ. Stephens and Christophers (1902), as members of the Malaria Committee of the Royal Society, made some observations on the same subject. They recorded the spleen rate as zero and found *A. subpictus* and *A. annularis* (*A. fuliginosus*). Dissections of 324 anophelines gave entirely negative results. It should be noted that *A. stephensi* was first described only in 1901 by Liston. James (1902) first recorded the presence of this species in Calcutta.

Rogers (1906) published an important paper dealing with malaria in Calcutta and its differentiation from 'seven-day fever'. He concluded (a) that malaria fevers in Europeans frequently originate in Calcutta in spite of the so-called 'endemic index of malaria' being nil, their main prevalence being from October to December, during the drying up of the rains and afterwards; whilst (b) seven-day fever (dengue) occurs every year in Calcutta in the early rainy season, and has a different seasonal incidence from malaria. Megaw (1907) published the results of one year's blood examinations made among out-patients at the Calcutta Medical College. Rejecting all the doubtful cases he records 165 malaria cases of true Calcutta origin (*P. vivax* 61, *P. malariae* 7, *P. falciparum* 97). B. Brahmachari (1909) carried out investigations in 1906-7 in Chitpur-Cossipore. He found four species of anophelines, viz., *A. subpictus*, *A. barbirostris*, *A. annularis* (*A. fuliginosus*), and *A. hyrcanus* (*A. sinensis*). Annandale, reported by Theobald (1908, 1910) and Brunetti (1907, 1912, 1920), found *A. stephensi* in the gardens of the Indian Museum. K. C. Bose (1912) remarks 'Burrabazaar in Calcutta is very bad with malaria cases'. He found anopheline larvæ in coconut shells in houses. U. N. Brahmachari (1912) found *A. sundanicus* (*A. ludlowi*) breeding in a tank in the Campbell Hospital compound. He also collected *A. stephensi* larvæ from an old tub. Alcock, Lloyd, Burkill, Bentham, Adie, Daniel, Annandale, James and Liston, and Brunetti collected 8 species of anophelines and 15 species of culicines, including *Aedes aegypti* and *Culex fatigans*,—these findings being recorded by Theobald (1908, 1910) and Brunetti (1907, 1912, 1920). Paiva (1912) carried out observations on the mosquitoes on the fringes of Calcutta. He recorded 6 species of culicines, including *Aedes aegypti* and 2 unclassified ones, and 2 anophelines, *A. subpictus* (*A. rossii*) and *A. sundanicus* (*A. ludlowi*)—(the last being a very doubtful observation from the nature of the breeding places he describes).

McGilchrist (1913) made an *Aedes* (*Stegomyia*) survey of the port of Calcutta from August 1912 to January 1913. James (1913) published a paper on the practicability of *Aedes* reduction in Indian seaports, with a view to the protection of India from yellow fever. In this connection he studied the conditions present in Calcutta and suggested the provision of a constant high pressure water supply as the first step that should be taken to reduce these mosquitoes. Christophers (1915) visited Calcutta to advise with regard to the best methods of carrying out a survey of the *Aedes* problem in Calcutta. He suggested an improved water supply, as an intermittent one leads to water storage and *Aedes* breeding. He proposed a complete scientific survey of all mosquito species in Calcutta, extending over five years.

In 1914-15 Dr. Nandy, reported by Christophers (1915), carried out an *Aedes* survey in two selected areas in Calcutta. Iyengar (1920) carried out

observations on the mosquitoes of Calcutta during 1917-19. He recorded *A. stephensi* breeding in the city and found spleen indices of from 2 to 10 per cent in the suburbs. He recorded 10 species of culicines, including *Aedes aegypti* and *Culex fatigans*. De (1923) collected 1,460 anopheline mosquitoes from districts III and IV of the city; he found only 1 per cent of them to be *A. stephensi*. The Health Officer of the city in his annual reports from 1922 to 1927 records death rates of from 1.1 to 1.6 per mille over a period of six years from malaria.

Basu (1930) published a paper dealing with observations on the density of *A. stephensi* breeding in Calcutta. Sur and Iyengar (1931) drew attention to the danger from an epidemic of malaria in Budge Budge and other suburbs of the town due to the introduction of *A. sundanicus* (*A. ludlowi*). Finally Covell (1932) made a thorough study of the whole problem of mosquitoes and malaria in Calcutta city, made recommendations, and published a comprehensive memoir on the subject.

RESULTS OF THE PRESENT INVESTIGATION.

I. ANOPHELES STEPHENSI.

(a) BREEDING PLACES.

During the four years, in the one square mile area around the Tropical School, in all 11,927 examinations for breeding places of *A. stephensi* were carried out, with 3,942 positive findings. This gives some idea of the density of *A. stephensi* breeding in the city. The total number of *A. stephensi* larvæ captured was 68,055; the highest numbers being found in July and the lowest in April. Chart II shows the frequency distribution of the larval catches month by month for the four years; the peak is very clearly in July.*

Twenty-one different types of breeding places were observed in the area concerned. The text-figure illustrates these graphically. Notes may be added on each.

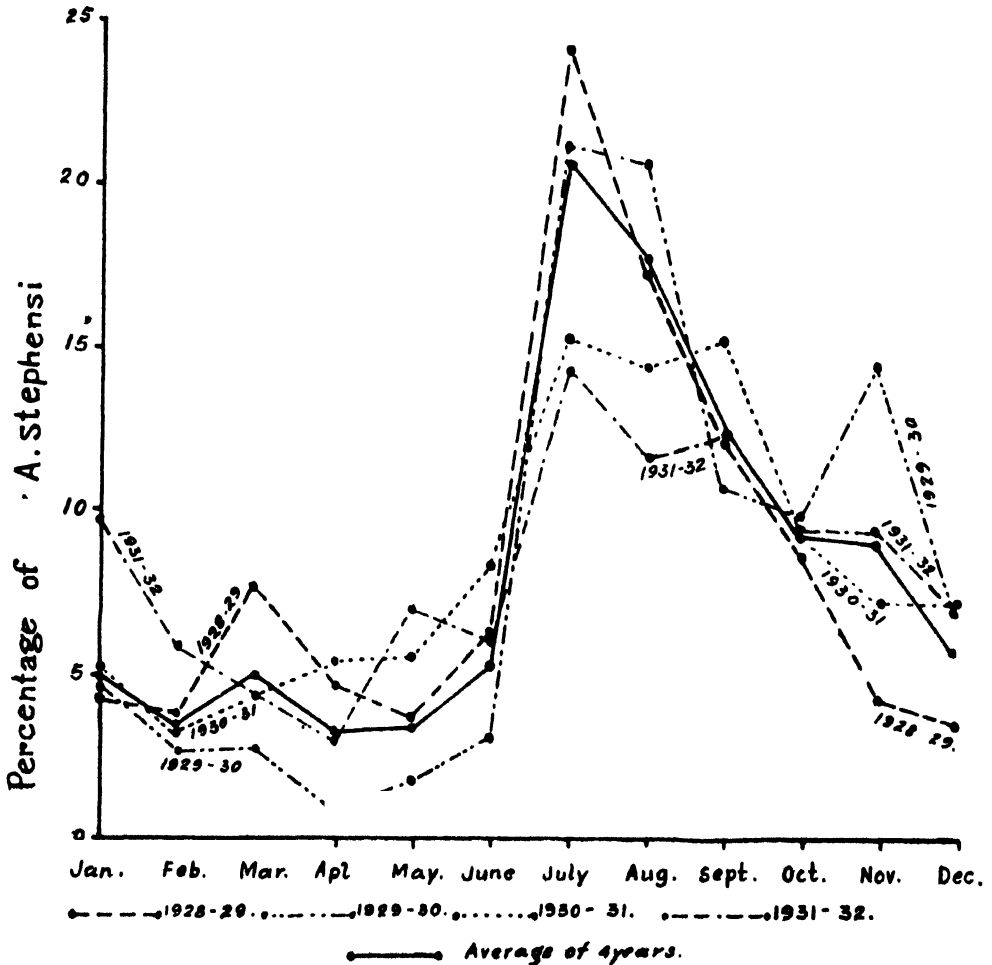
'Chowbachas' (Fig. 1). These are masonry reservoirs for the storage of filtered water, varying in size from a capacity of 50 to 500 gallons or more. They are mostly found in pucca buildings and only a few in bustis, since a piped water supply to busti houses is a recent innovation only. Each filtered water connection has at least one such reservoir, if not more. In 1932 these connections numbered 50,528. During the four years 4,148 examinations of *chowbachas* were carried out for larvæ of *A. stephensi*, and 1,549—or 37.3 per cent—gave positive findings.

Cisterns (Fig. 2). These are made of galvanised iron sheets of standard thickness, and are mostly used as reservoirs for the storage of unfiltered water for flushing latrines. Every house with a sanitary privy has at least one on its roof. Their capacity is proportional to the number of latrines in the house, as the Corporation rules demand a capacity of 60 gallons per latrine. Sometimes four or five may be on one roof.

*The percentage in the vertical axis of the chart is the percentage month by month of the total larval catch for the year.

In the old type of Indian houses the kitchens used to be on the ground floor in the same block as the dwelling rooms, and the middle class Indians, instead of using gas or electricity, burnt coke and wood for cooking purposes. This led to an intolerable smoke nuisance in the city. As a result of repeated warnings by the Smoke Nuisance Committee, which pointed out the increasing

Chart II



Seasonal prevalence of *A. stephensi* breeding in Calcutta over four years, and average of the same.

number of cases of diseases of the lungs in Calcutta, and the damage done to the permanent buildings by coke and wood smoke, the inhabitants of these buildings have been gradually shifting their kitchens to the roofs of their houses of recent years. This means an increased demand for filtered water on the roof, but, as the pressure of the filtered water supply is only a few feet,

people collect the filtered water in a *chowbacha* (masonry reservoir) on the ground floor level and thence pump it by an electric pump into a cistern on the roof. On enquiry we were informed by the dealers in plumbing material and goods that the sale of pumps and cisterns is rapidly increasing throughout the city. Thus the introduction of roof kitchens may lead to smoke abatement, but unless a sufficiently high pressure and a continuous supply of filtered water be provided, this will add greatly to the danger of *A. stephensi* breeding in the city.

Each unfiltered water connection means one or more cisterns, and in 1932 the total number of unfiltered water connections in the city was 41,702. Most of the cisterns in Calcutta have broken lids or are without lids; each has a side hole for overflow of surplus water, whilst many have holes made by the test officer to test the thickness of the galvanised iron sheets. Thus the entry of mosquitoes for oviposition is easy. Covell (1932) has recommended a design of mosquito-proof cistern for use in Calcutta, as he did in Bombay.

A. stephensi were found breeding profusely in cisterns on the roofs of from one- to five-storeyed houses. During the four years, out of 1,983 examinations 628—or 31·6 per cent—gave positive results.

Taken together, these observations show how very important sources of *A. stephensi* breeding are these *chowbachas* and cisterns.

Wooden barrels (Fig. 3). 1,983 examined, 32·6 per cent positive.

Earthen 'handis' (Fig. 4). 1,240 examined, 29·3 per cent positive.

Earthen tubs (Fig. 5). 265 examined, 26·4 per cent positive.

Earthen jars (Fig. 6). 1,064 examined, 30 per cent positive.

Kerosene tins (Fig. 7). 219 examined, 29·2 per cent positive.

Iron tubs (Fig. 10). 809 examined, 28·3 per cent positive.

Iron barrels (Fig. 15). 7 examined, 1 positive.

These receptacles are used to a greater or less extent by the poorer classes for storing filtered water; many of them are discarded articles in which rain water collects and provides suitable breeding sites for *A. stephensi*. Some of the earthen tubs are used as garden tubs in which rain water collects, whilst some of the iron tubs are used as fire buckets in buildings, from many of which larvæ of *A. stephensi* were collected.

Tin mugs (Fig. 8). 80 examined, 16 positive.

Iron frying pans (Fig. 9). 65 examined, 20 positive.

Wooden boxes (Fig. 11). 113 examined, 40 positive.

Glass phials (Fig. 13). 6 examined, 2 positive.

Motor mud-guards (Fig. 14). 7 examined, 1 positive.

Tin boxes (Fig. 16). 5 examined, 1 positive.

Teapots (Fig. 17). 6 examined, 4 positive.

Cups (Fig. 19). 8 examined, 1 positive.

Deserted fireplaces (Fig. 20). 3 examined, 2 positive.

Most of the above articles were found discarded, and water had accumulated in them.

Pitch barrels (Fig. 12). 128 examined, 40 positive or 38·2 per cent. These were found mostly by the roadside. After the pitch has been used up in repairing the roads, the empty barrels are left lying about before they are removed; rain water accumulates in them and suitable breeding sites are formed.

Garden fountains (Fig. 18) are not very common in the area.

Pucca drains (Fig. 21), when blocked by dirt and with stagnant water in them, form suitable breeding sites. Out of 21 examined 10 showed larvæ.

TEXT-FIGURE.

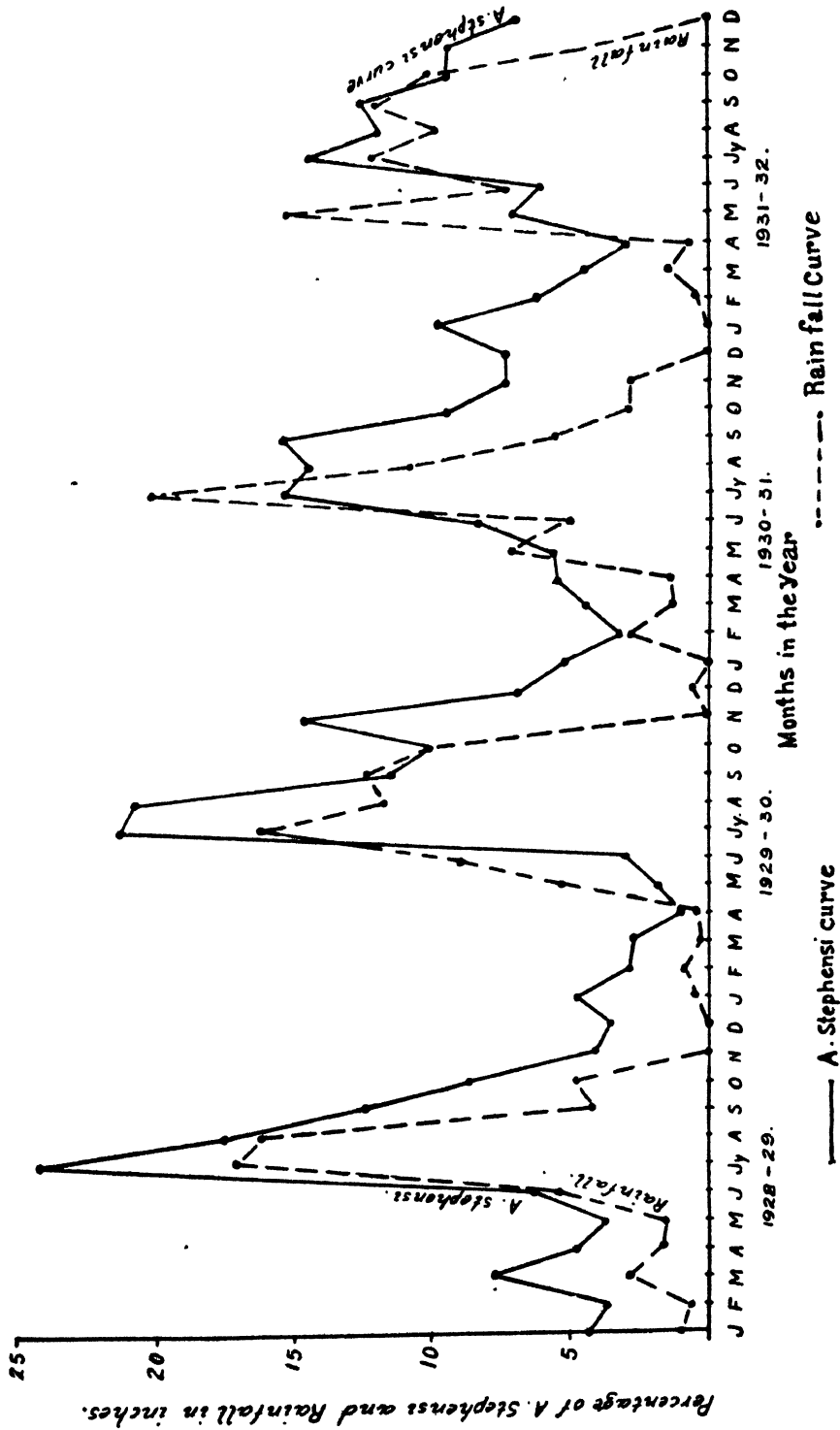


Types of breeding places of *Anopheles stephensi* in Calcutta.

A. stephensi larvæ were not found at any time during the four years in the tank in College Square.

In Howrah and the northern suburbs along the river there are no sanitary privies; hence roof cisterns are rare. Here *A. stephensi* was found breeding in chowbachas and other receptacles for water storage. In Bally and Kutrum

Chart III

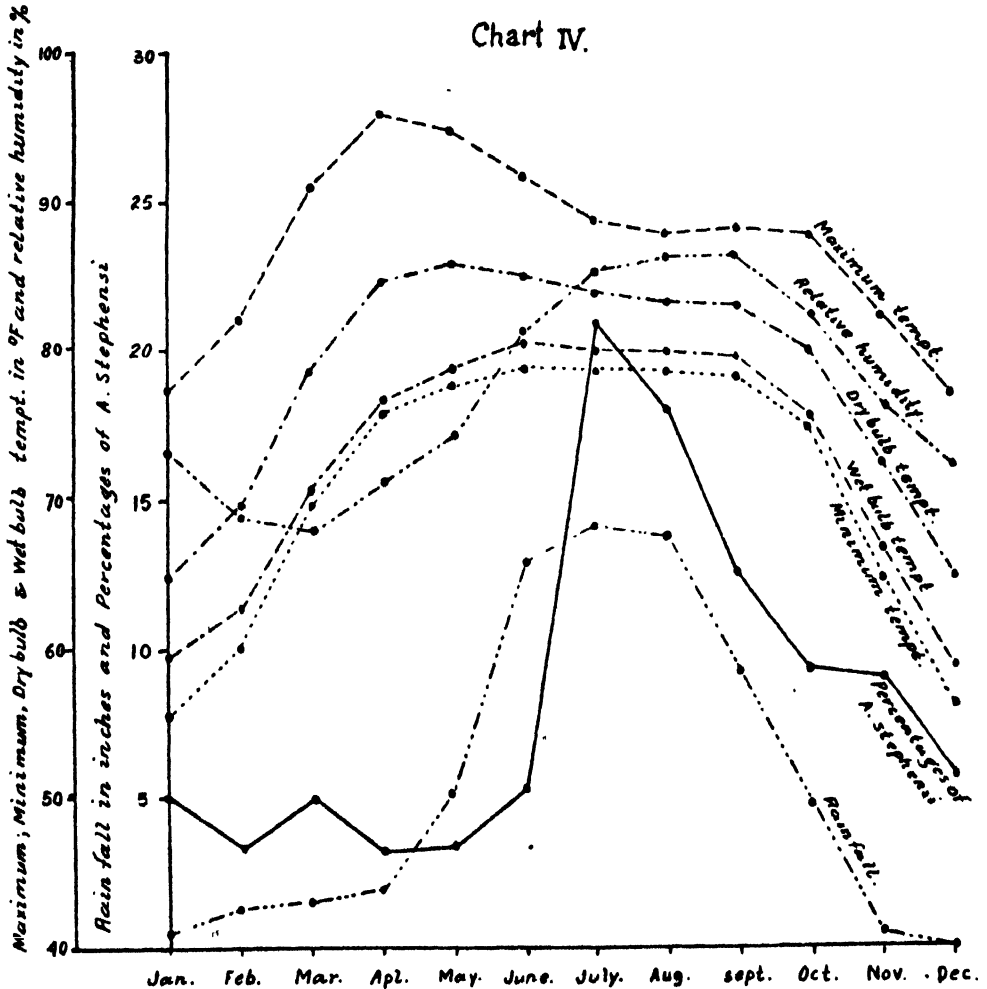


A. Stephens breeding and rainfall in Calcutta, over four years.

there are a large number of brickfields and small pucca ditches for the storage of water; here *A. stephensi* was found breeding profusely.

(b) RELATIONSHIP TO METEOROLOGICAL CONDITIONS.

Chart III shows the monthly seasonal incidence of *A. stephensi* over the four years and its correlation with rainfall. It will be seen that the two coincide very closely.*



A. stephensi breeding and meteorological conditions in Calcutta.

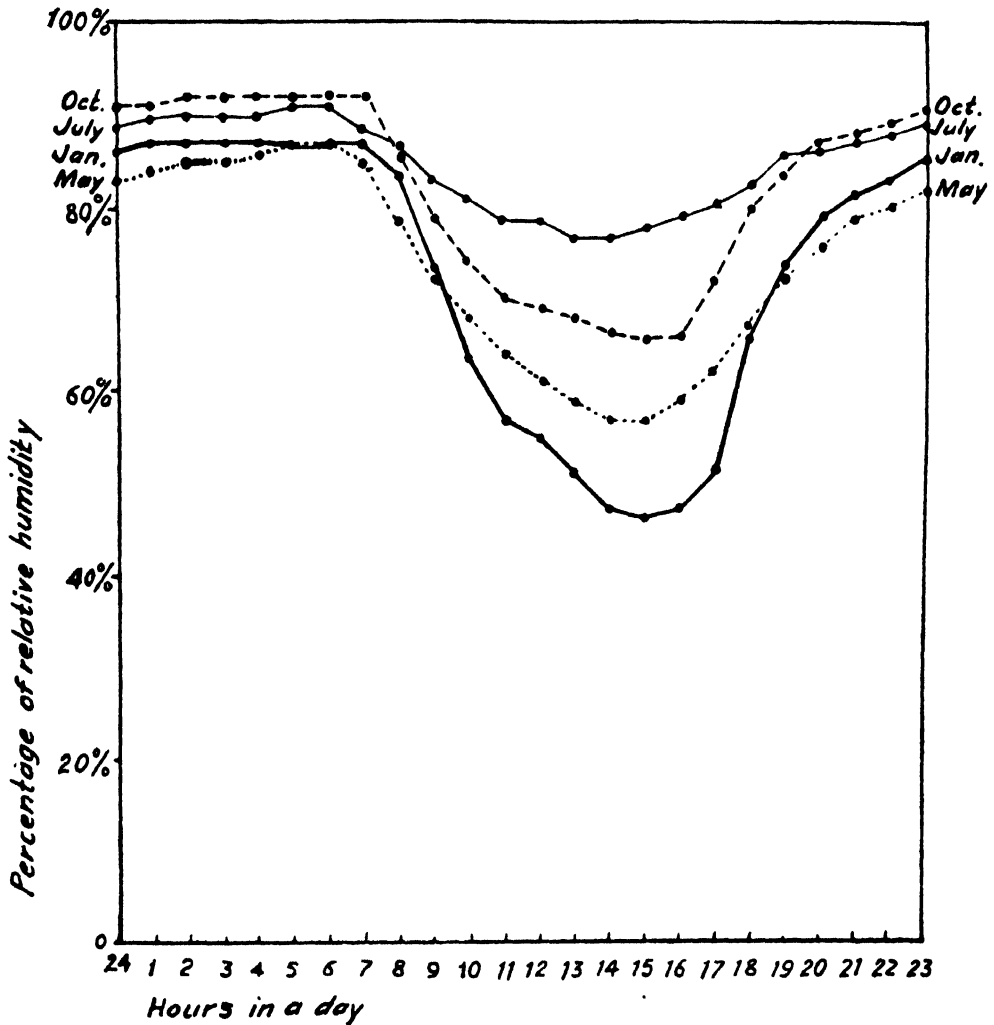
Chart IV shows the correlation between *A. stephensi* breeding and meteorological conditions in Calcutta. The *A. stephensi* curve shows the averages derived from the figures for four years, whilst the meteorological curves are the means calculated from twenty years' observations at Alipore Observatory. The curves for rainfall, average minimum temperature, wet bulb temperature, and

* The figures on the vertical axis are percentages of the total *A. stephensi* larvae collected during the year, for each month in turn.

average relative humidity coincide with the curve for *A. stephensi* breeding. The peaks of the average maximum temperature and average dry bulb temperature coincide with the fall in the *A. stephensi* curve.

Chart V shows the variation in relative humidity at different hours of the day in different seasons of the year. These values are again calculated from

Chart V



Relative humidities hour by hour in Calcutta at different seasons of the year; means of twenty years.

the Alipore observations for twenty years and can therefore be relied upon as being normal values. The months of January, May, July and October respectively may be taken to be those most typical of the cold weather, the hot weather, the monsoon season, and the dry period after the rains.

II. *Aedes Aegypti*.

During the same four years the seasonal breeding of *Aedes aegypti* was observed in the same area. Out of 11,927 examinations of suspected breeding places 4,938 gave positive results; the total number of larvæ captured being 135,489. The greatest intensity of breeding was observed in July and August, and the lowest in February and April. These results are shown in Chart X. The chief sites of breeding corresponded more or less with those of *A. stephensi*, being *chowbachas* (37 per cent of observed breeding places), cisterns (15 per cent), wooden barrels, earthen *handis*, earthen jars, kerosene tins, and in general any receptacles for water storage.

III. *Culex fatigans*.

The breeding of *Culex fatigans* was studied in the same area for two years. Out of 4,339 examinations of suspected breeding places 332 gave positive results, the total number of larvæ captured being 6,104. The greatest intensity of breeding was observed in November, and the lowest in July. These results are shown in Chart XI. The chief breeding places were again water storage receptacles—*chowbachas* (50 per cent of observations), cisterns, wooden barrels, earthen *handis*, tubs, earthen jars, and drains.

IV. *Anopheles stephensi* and malaria.

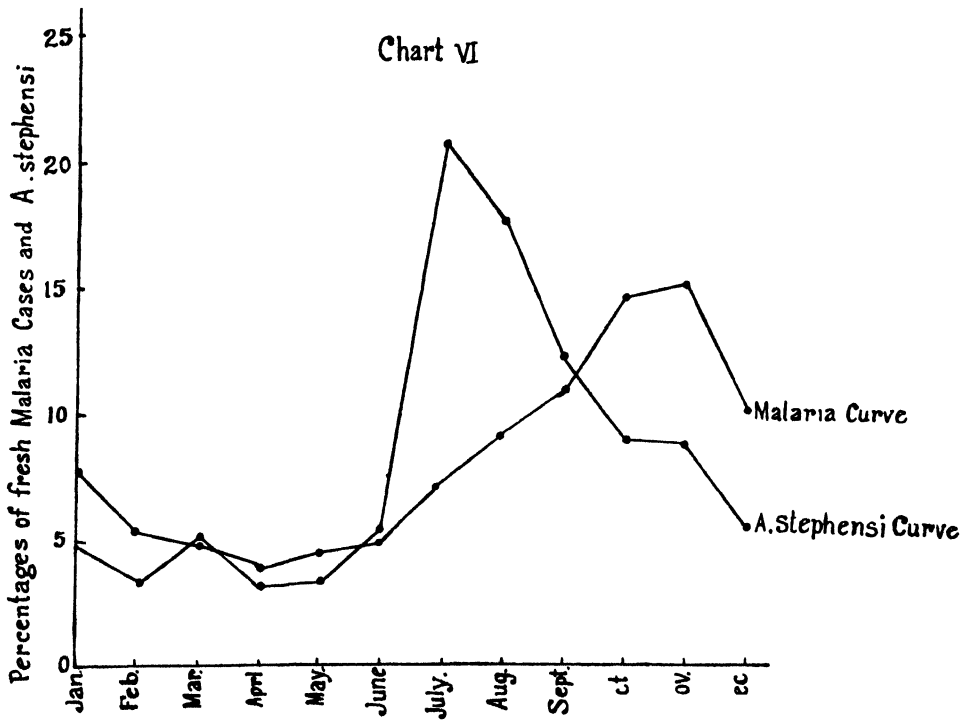
It is a little difficult to say to what extent malaria is at present endemic in Calcutta city. During the last two years the senior author has encountered four instances in which malaria was undoubtedly contracted in the city; one the night sister to the Carmichael Hospital for Tropical Diseases, one a resident in a third floor flat in Chowringhee, and two residents in Galstaun Mansions in Park Street. Rogers (1906) considers that Europeans in Calcutta not infrequently contract malaria in the city. Covell (1932) has shown that splenic indices among municipal school children, aged 6 to 10 years, varied from 0 to 3.8, with parasite indices of from 0 to 15 in different wards of the city. Though agreeing that cases which are undoubtedly of local origin occur, Covell thinks that a large number of infections are acquired by persons who go out of Calcutta to malaria infested areas in the *mofussil* during the Puja holidays (September-October).

The problem is further complicated by immigration into the city from the rural areas of Bengal. The population of Calcutta is a constantly changing one. The census report of 1921 states that no less than 664 residents of Calcutta per mille had been born elsewhere and had come into the city from outside. The following figures from that report and from Bentley (1930) are instructive :—

Immigration from districts into Calcutta, 1921.	Number of immigrants, 1921.	Death rates from malaria in the districts concerned. 1928	Fever indices in the same districts 1928.
Hooghly	47,092	9.3	53.7
Midnapore	36,082	6.5	37.9
Burdwan	20,627	8.2	51.0
Jessore	9,548	21.2	44.3

The list might be continued almost indefinitely. It is therefore certain (a) that many residents of Calcutta acquire malaria from infections contracted in the *mofussil*, and (b) that malaria is imported wholesale into Calcutta city by immigrants coming into the city from the endemic areas in the Bengal *mofussil*.

With regard to the seasonal incidence of malaria in Calcutta during the year, there is a good deal of information available. There are the data collected by Knowles and Senior White (1930). The authorities concerned at the Presidency General Hospital, the Indian Military Hospital, and the Medical College Hospital have also very kindly furnished us with their figures for in-patients for the years 1928-31 inclusive. The figures for these four reports taken together total 2,871 cases, and the percentage of the total number of cases seen monthly is shown in Charts VI, VII, and IX. The peak month is November, with 15.2



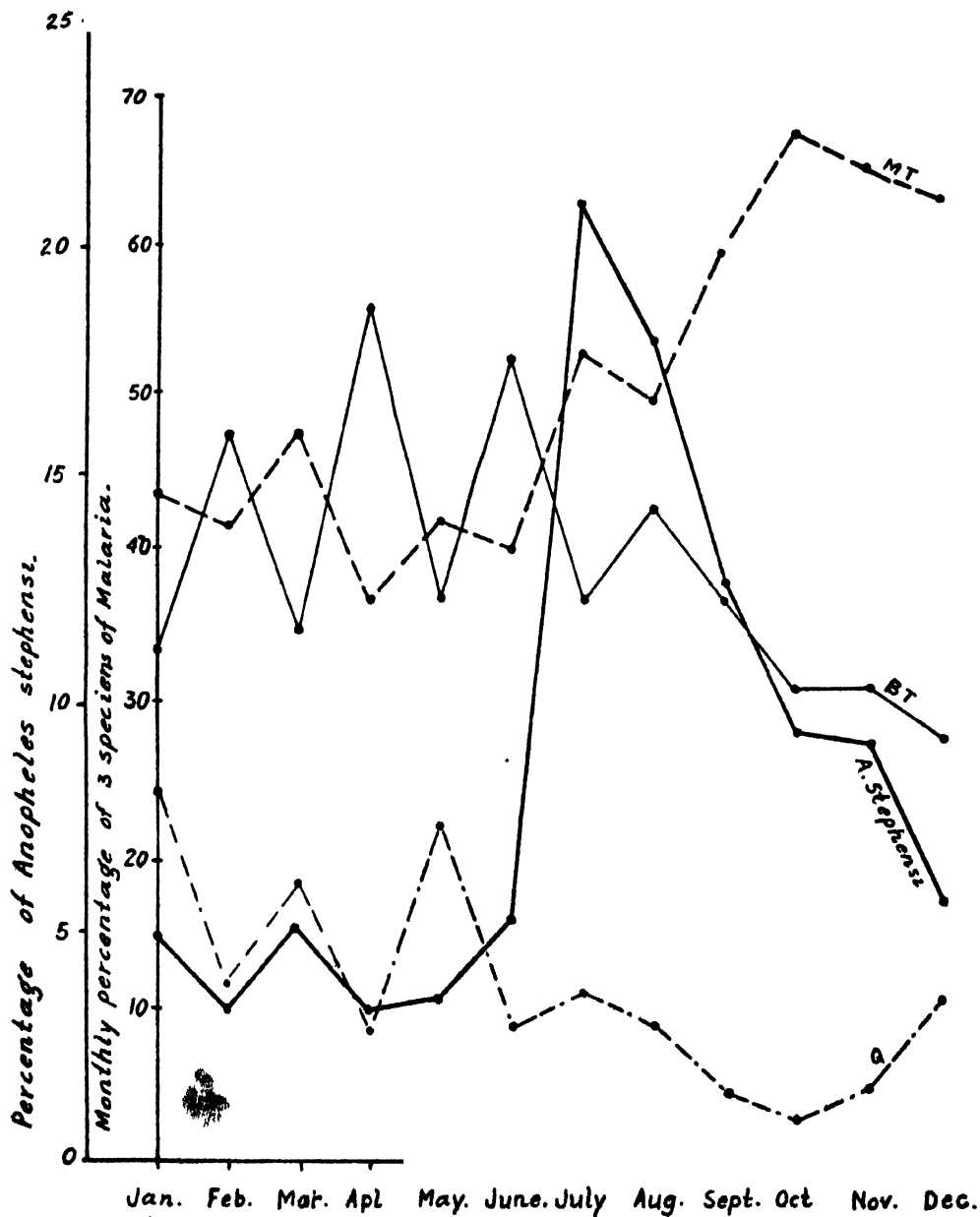
Incidence of fresh malaria cases and *A. stephensi* breeding in Calcutta during the year

per cent of all cases of malaria seen throughout the year, whilst no less than 51.5 per cent of all malaria cases seen occur in the period September-December. Very little malaria is seen in the period March-May.

Chart VII shows the distribution of malaria in Calcutta throughout the year by species (1,238 cases in all), taken from the memoir by Knowles and Senior White (1930), together with the *A. stephensi* curve. It will be seen that the peak of *stephensi* incidence in July does not correspond with the peak of

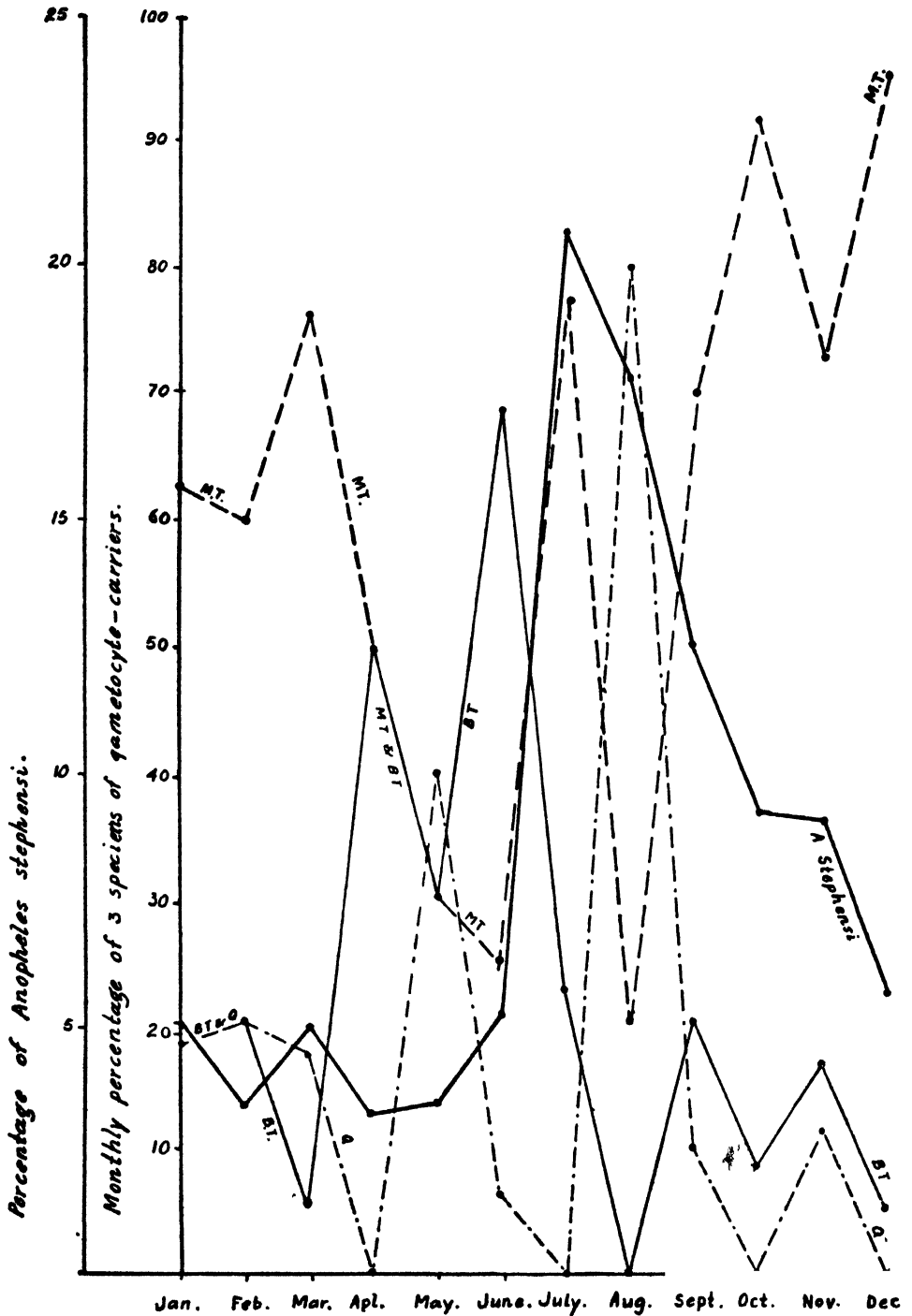
incidence for *P. falciparum* in October, of *P. vivax* in April, or of *P. malariae* in January.

Chart VII



Incidence of malaria due to the three different species of parasites, and of *A. stephensi* breeding in Calcutta during the year

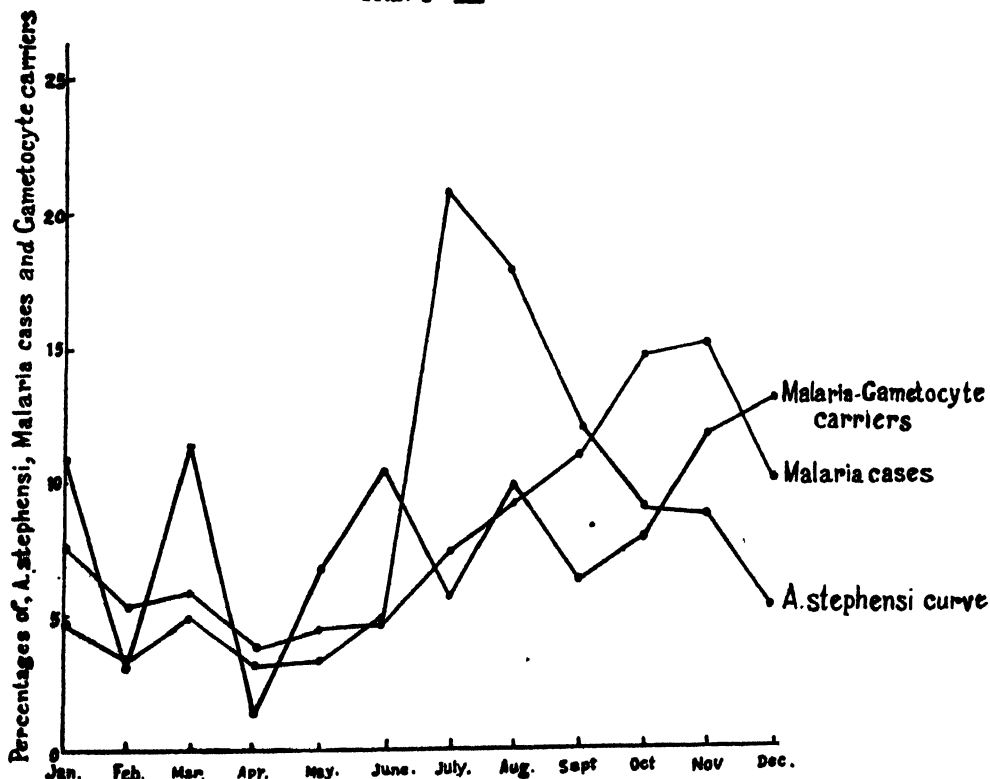
Chart VIII



Incidence of malaria gametocyte carriers, due to the three species of parasites, and of *A. stephensi* breeding in Calcutta during the year.

Chart VIII is taken from the malaria memoir by Knowles and Senior White (1930). It is based upon four years' observations in the out-patient department of the Calcutta School of Tropical Medicine, and shows the relative monthly distribution of gametocyte carriers of the three species of malaria parasites, with the *stephensi*-density curve superimposed. It will be seen that the *stephensi* curve fails to correspond with the maximum incidence of carriers of *P. vivax* (June), *P. malariae* (August), and *P. falciparum* (December).

Chart IX



Incidence of total malaria, of gametocyte carriers, and of *A. stephensi* breeding in Calcutta during the year.

Chart IX shows the failure of the peak of the *stephensi* curve (July) to coincide with the maximum incidence of malaria cases (November) or of gametocyte carriers of the three species (December).

We have now the following facts recorded :—

(i) *Anopheles stephensi*, a notorious carrier of malaria, is pullulating in almost every other receptacle for water storage in Calcutta city.

(ii) Malaria infection *en masse* is constantly being imported into Calcutta city with immigrants from the endemic areas in Bengal and by visitors to such areas.

(iii) Yet there is but little endemic malaria in the city itself.

How are these three findings to be correlated?

In order to clarify matters, it may be best to put what follows into the form of two questions and answers:

(i) Is the Calcutta strain of *A. stephensi* capable of transmitting malaria?

The answer is emphatically: Yes. In hundreds of experimental feeds of adult females of this species bred out from larvæ collected locally upon gametocyte carriers, we have seen the salivary glands packed with sporozoites.

(ii) Are the climatic conditions unsuitable for malaria transmission?

The answer is emphatically: No. The Malaria Transmission Enquiry at the Calcutta School of Tropical Medicine commenced work in April 1931. Its work has been very largely carried out in a specially constructed air-conditioning cabinet which has been described elsewhere (Knowles, 1932). In this cabinet the temperature can be adjusted to any range between 50° to 100°F., and the relative humidity to any range between 50 and 100 per cent. Up to date, 6,064 laboratory-bred female *A. stephensi* have been fed upon gametocyte carriers (of all three species of malaria parasites), kept in the air-conditioning cabinet at five different ranges of temperature and relative humidity corresponding to the five seasons of the year in Calcutta and serially sectioned after different periods of time. The results are summarised in Table I.

TABLE I.

Rates of infection of Anopheles stephensi with the three species of malaria parasites, under the different climatic conditions prevailing in Calcutta city.

Period.	Months.	AVERAGE MEAN TEMPERATURE.		Relative humidity.	PERCENTAGE OF INFECTION IN <i>A. stephensi</i> WITH			Longevity of <i>A. stephensi</i> .
		Dry-bulb.	Wet-bulb.		M. T.	B. T.	Q.	
Cold weather.	December.	66·2°F.	60·0°F.	72	72	23·5	?	73 per cent (14-20 days).
	January.							
	February.							
Spring	March.	81·3°F.	73·3°F.	68	25	0	11	27 per cent (7 days).
	April.							
Hot weather.	May 1st to June 15th.	85·5°F.	79·1°F.	76	22	?	0	51 per cent (7-13 days).
Monsoon	June 16th to September 15th.	83·4°F.	79·7°F.	85	30	51	?	63 per cent (8-14 days).
Post-monsoon.	September 16th to November 30th.	77·7°F.	73·0°F.	80	64	41	44	50 per cent (7-9 days).

In another series of experiments 6,326 laboratory-bred females of *A. stephensi* were fed upon gametocyte carriers (of all three species of malaria parasites). These insects were then exposed to different ranges of temperature and relative humidity—ranging from 50°F. and 50 per cent humidity to 80°F. and 90 per cent humidity in the above-mentioned cabinet and the survivors dissected after different periods of time. The result may be summarised as follows :—

(a) Infection with *P. vivax* occurs at 60° to 80°F., with humidities of 80 to 90 per cent, and the heaviest salivary gland infections were obtained at 80°F. This corresponds roughly to the Calcutta meteorological conditions from 1st May to 30th November.

(b) So far we have not been able to obtain salivary gland infection with *P. malariae*.*

(c) That infection occurs with *P. falciparum* at ranges between 70° and 80°F., and humidities between 50 to 90 per cent, and, further, salivary gland infection is seen very frequently within this range. This would correspond to the Calcutta meteorological conditions between 16th June, *i.e.*, after the onset of the monsoon, to 30th November.

We have further the undoubted fact that malaria transmission *does* occur in Calcutta city, and is presumably due to *A. stephensi*.

Conditions could not present a greater contrast in this matter than those present in Bombay and Calcutta, respectively. The report by Covell (1928) gives an admirable account of malaria in Bombay. The city has been notorious for its malaria incidence for years, and, as Covell shows, *A. stephensi*—breeding chiefly in wells—is the one and only species responsible for this.†

We may put forward three possible reasons for the discrepancy in Calcutta :—

(i) In spite of the fact that larvæ of *A. stephensi* are to be found in profusion throughout the city, yet adults of this species are curiously difficult to capture. It may be remarked that De (1923) found only one per cent of 1,460 adult anopheline mosquitoes from districts III and IV of the city to be *A. stephensi*. Adults *must* be present in considerable numbers to account for the profuse breeding which is taking place, but whether they are zoophilous rather than androphilous has still to be determined. Some students of *A. maculipennis*—Roubaud in France, Wesenburg-Lund in Denmark—ascribe the great diminution in endemic malaria during the last century in their respective countries to gradually acquired zoophilism of this species. Roubaud (1921) even goes so far as to distinguish zoophilic and androphilic races of this species, basing his distinction on the number of serrations on the maxillæ. The absence

* There is something 'queer' about quartan malaria. The matter has been fully discussed elsewhere—Knowles and Senior White (1930)—so need not be commented on further here. One almost wonders whether the transmitting vector of this species may not be an insect more personally intimate with man than a mosquito. It is admittedly difficult to infect a mosquito with this species.

† It must be added that, since Colonel Covell's report was published, the municipal authorities in Bombay have taken vigorous measures to 'clean up' the situation, and it is now very much better than it was. In fact the senior author, when recently in Bombay, was informed by a leading medical authority in the city that there is now very little endemic malaria in Bombay itself.

of adults may be more apparent than real, and the whole subject is now under investigation.

(ii) There is a distinct want of correlation between the peak of incidence of *A. stephensi* breeding and of cases of malaria and gametocyte carriers. This has been brought out in Charts VIII and IX. Chart VI shows the general want of relationship between the incidence of malaria in Calcutta and *A. stephensi* breeding. The peak for malaria incidence is in November, a period at which *stephensi* breeding is falling very rapidly. Calcutta has definitely a much more marked cold weather than Bombay has and the difference in climatic conditions may account for the prevalence of malaria in Bombay and its relative absence from Calcutta. On the other hand many places in Bengal, with much the same climate as that of Calcutta, are notoriously malarious.

(iii) As we have seen, the transmitting season is between June and November. At this season of the year many, or most, of the inhabitants of Calcutta live, eat, work and sleep under an electric fan. The electric fan indeed is probably the best anti-mosquito measure yet introduced by science. On the other hand, electric fans must be just as much in use in Bombay as in Calcutta.

In brief the present (possibly only apparent) freedom of Calcutta from malaria cannot be taken as any guarantee for the future. The state of affairs reported in Colonel Covell's 1932 report is sufficiently serious, and, in addition to *A. stephensi*, *Anopheles sundaicus* (*A. ludlowi*)—an even worse carrier—is now breeding within the municipal limits, and is acclimatising itself to breeding in waters of less and less salinity. The future is very far from certain.

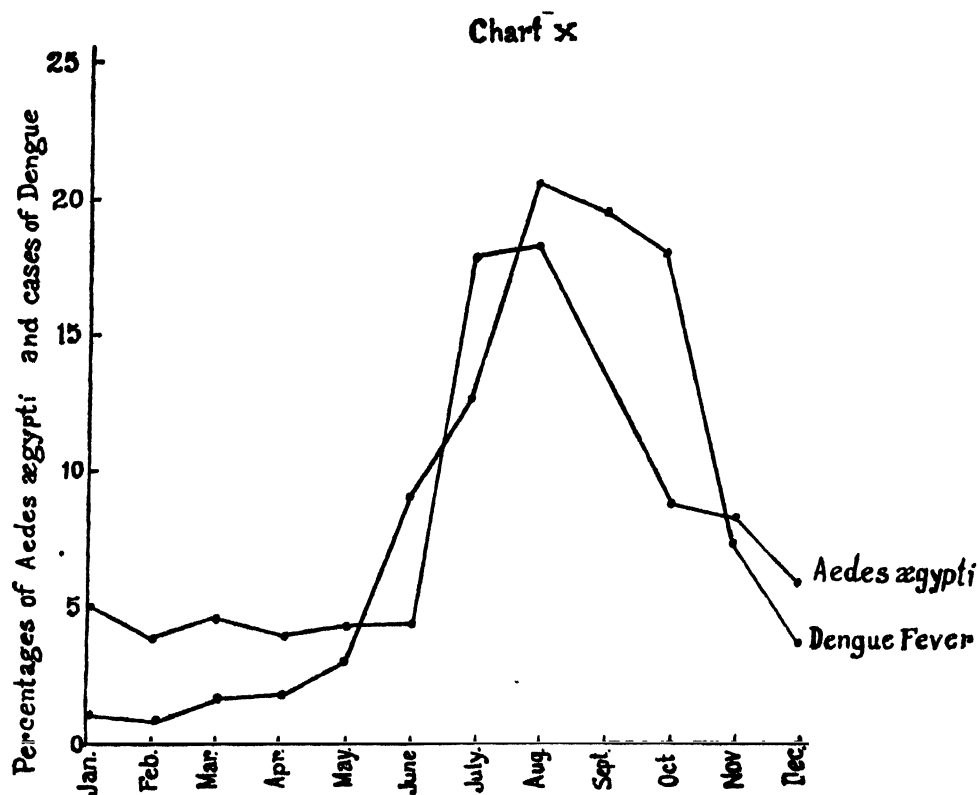
V. *Aedes aegypti* AND DENGUE.

When we turn from *Anopheles stephensi* and malaria to the problem of *Aedes aegypti* and dengue, we come to a completely different state of affairs. Chart X shows (a) the seasonal prevalence of *Aedes aegypti* breeding, as observed in Calcutta over a period of four years, and (b) the monthly admissions for dengue to five of the biggest hospitals in Calcutta—1,482 cases in 4 years. Here it will be seen that there is a very close relationship. The peak for *Aedes* breeding is in July-August, and that for new cases of dengue in August-September.

Dengue is a disease which is not associated with any mortality rate (except for the recent 1928 outbreak in Greece, where, for some reason, the disease caused an appreciable mortality). On the other hand, it is a very crippling disease, and the sufferer is confined to bed usually for at least seven to ten days, and often a fortnight or longer absent from duty. Chart X shows what Calcutta city suffers for its neglect of 'the mosquito menace'. Every year dengue is prevalent from July to November, and every third year or so the disease assumes epidemic—or even severely epidemic—proportions. In heavily epidemic years something like 30 to 40 per cent of the population may be affected, and such epidemics must cost the commercial industries of Calcutta (and the Government of Bengal) crores of rupees. Unlike the case with yellow fever, immunity to dengue appears to be established only slowly and gradually. The history of a new-comer to Calcutta is usually that he has three or four attacks of dengue in his first five or six years' residence in the city; after that renewed infections in the autumn cause only a feeling of malaise and rheumatic pains.

The view that dengue is due to a leptospira organism must now be abandoned. It is fully discussed elsewhere (Knowles and Das Gupta, 1924), so need not be commented on here; the recent evidence all goes to show that dengue is due to a filterable virus, and that it presents many analogies to a mild type of yellow fever.

Before it became clear that dengue is due to a filterable virus, we devoted some little attention to the possibility of its being a spirochætal disease, and it may perhaps be permissible here to give a short note on this work. During



Incidence of breeding of *Aedes aegypti* and of fresh cases of dengue in Calcutta during the year.

the epidemic autumn season of 1927 in all 46 wild female *Aedes aegypti* were captured and dissected, the gut and salivary glands being searched for leptospira-like organisms; in 1928 the number similarly examined was 105; in 1930 it was 52. During the autumn epidemic season of 1927 in all 70 laboratory-bred females of *Aedes aegypti* were fed upon patients in the early phase of an attack of dengue, and were dissected subsequently at intervals of from 1 to 13 days, being kept at room temperature. During the epidemic autumn season of 1928 the number similarly fed and dissected was 46. In all, we have examined 203 female adults captured during the dengue season in the wild state, and 116 fed upon dengue patients during the epidemic season. Nothing suggestive of a

micro-organism responsible for the disease was found, but it may be of interest to record such organisms as were found. These were as follows :—

(i) From the gut of five specimens a bacillus was isolated with the morphological and cultural reactions of the *Bacillus metacolooides*.

(ii) In the gut of four an infection with a herpetomonad, which was apparently *Herpetomonas culicis*, was found.

(iii) In two a filamentous non-motile bacillus, which we were unable to cultivate.

(iv) In one Rickettsia-like forms in the epithelial cells of the gut and in the salivary glands.

(v) In the gut of one very pleomorphic bacilli with metachromatic granules, resembling diphtheroid bacteria.

(vi) In the gut of one a bacillus with the cultural reactions of the *Bacillus lactis aerogenes*. (This finding was of interest, as a dengue-like disease has been described as due to infection of the blood stream by this bacillus. The finding was, however, an absolutely isolated one.)

(vii) The gut of one mosquito dissected was packed with spirochaetes, which, however, we were not able to cultivate under aerobic and anaerobic conditions, and could not identify.

(viii) *Bodo* sp. was present in the gut of one mosquito.

(ix) A small, very actively motile cocco-bacillus was present in the gut of one mosquito, and what was apparently the same organism in the salivary glands of another. Aerobic and anaerobic cultures, however, were unsuccessful.

(x) The gut of one mosquito contained very numerous tiny translucent crystals resembling Charcot-Leyden crystals, but we were unable to obtain a fixed and stained specimen.

It is clear that these findings merely represent accidental infections which the mosquitoes concerned may acquire during life. They have no bearing on the dengue problem, but they illustrate very well the difficulties with which workers on insect-transmitted diseases may be faced.

VI. *CULEX FATIGANS* AND FILARIASIS.

Chart XI shows (a) the percentage of breeding of *Culex fatigans* as observed, month by month throughout the year, for two years in the area of one square mile around the Tropical School, and (b) the distribution month by month throughout the year of 2,796 new cases of filariasis seen at the Calcutta School of Tropical Medicine in four years. For the latter figures we are very much indebted to Dr. S. Sundar Rao, Darbhanga Research Scholar on Filariasis at the School.

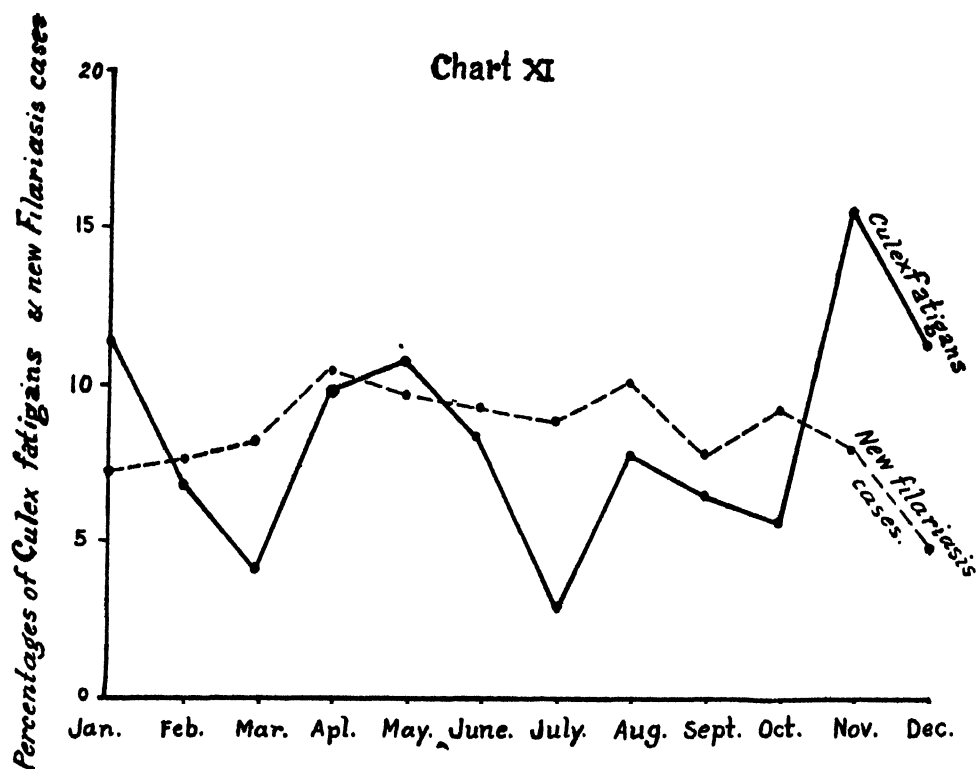
It cannot be said that the two curves show any very close approximation. The whole of the work at the School on the epidemiology of filariasis in India by Acton and Sundar Rao has been reviewed elsewhere (Knowles, 1934), and need not be repeated here. Their main conclusion, however, is that India may be divided into three main geographical areas with regard to infection by *Wuchereria (Filaria) bancrofti*, viz.:—

(i) *Arcas in which filariasis is hyperendemic, with microfilaria rates of 20 per cent and upwards.* Here *Culex* breeding is going on throughout the year; infestation with such mosquitoes is prevalent at all times; climatic conditions are suitable for filariasis transmission for nine months or even more of the year. Owing to constant blocking of the superficial lymphatics by immature worms injected by mosquitoes, the chief filarial lesion in such areas is elephantiasis.

(ii) *Arcas in which filariasis is moderately endemic, with microfilaria rates of from 10 to 20 per cent.* Here transmission is only possible for some four or five months of the year, or less; *Culex* breeding is less intense; and infections

less frequent. Only occasional doses of immature worms are injected by infected mosquitoes, and these usually succeed in reaching the deeper lymphatics before they can do much damage. In such areas the chief manifestations of filariasis are filarial fever, hydrocele, chyluria, lymph scrotum, acute funiculitis of the spermatic cord, and above all acute lymphangitis and lymphadenitis. Elephantiasis is a late, and only occasional, manifestation.

(iii) Areas in which filariasis is only slightly endemic, with microfilaria rates of under 10 per cent. Here only a few immature worms are injected at rare intervals into man by infected mosquitoes. These pass rapidly to the



deeper lymphatics without causing obstruction. The chief lesions seen are filarial fever and sometimes chyluria. Elephantiasis is rare or absent.

Calcutta has a general microfilaria rate of about 9.5 per cent (as determined by Dr. Sundar Rao over many years of observation of general in-patients suffering from other diseases in the Carmichael Hospital for Tropical Diseases). It therefore falls within group (iii) as outlined above. It has been shown by Rao and Iyengar (1930) that it is especially during the monsoon period that conditions are most favourable for transmission of filariasis. At this time not only do the embryos develop more rapidly in the mosquito than at other times, but they also develop in larger numbers and a much heavier percentage of fed mosquitoes become infective than at other times. Chart XI shows that *Culex*

breeding during the monsoon period of July to October is at a low ebb, this coinciding with the period most favourable for filariasis transmission; on the other hand when *Culex* breeding reaches its maximum density, in November, conditions for filariasis transmission are rapidly becoming unfavourable. Further, it is difficult or impossible to determine the incubation period in filarial infections in man, and the want of correlation of the two curves in Chart XI may be explained by this. What the two curves do suggest is that *some* transmission occurs throughout most of the year, but there is no epidemic wave, as in the case of *Aedes aegypti* and dengue.

We do not mean to suggest for one moment that filariasis is not a serious problem in Calcutta city. It is a *most* serious economic problem in Calcutta. It is almost incredible what a large proportion of the Anglo-Indian and Hindu population of the city are infected. Anglo-Indian girls, stenographers, typists, employees in the telephone exchanges are infected in large numbers, and the disease causes them much loss of pay and of employment. The better and middle class Hindu population is also heavily infected—at the Calcutta Tropical School it is found among assistant research workers, laboratory assistants, clerks, and the like. The disease causes an immense amount of economic suffering and hardship among certain classes in Calcutta, and it constitutes a problem perhaps even more serious than that of dengue in the city.

REMEDIAL MEASURES RECOMMENDED.

We may now pause to review the evidence which has been collected in this paper. Malaria is not apparently a very serious danger to Calcutta city, but we have already one virulent mosquito carrier—*Anopheles stephensi*—breeding in almost every other water storage receptacle in the city, together with the recent introduction of a second, and even more virulent carrier, *Anopheles sundaicus* (*A. ludlowi*). The future is quite uncertain and it would not be safe to anticipate. Further enquiry is urgently called for (and is at present in progress).

Dengue is a perpetual nuisance in Calcutta and from time to time it assumes a severe epidemic form. The mosquito which transmits the disease is known, its breeding places in the city have been described, and its eradication ought to be possible. Dengue must cause a very big financial loss to the commercial industries of Calcutta annually.

Filariasis in Calcutta city is a disease which especially affects the Anglo-Indian and Hindu communities. It is a cause of very much suffering and economic loss among the poorer Anglo-Indian and among Hindu communities. The mosquito which transmits it can be eradicated if measures be taken against the other two species responsible for mosquito-borne diseases in Calcutta.

The cure for this state of affairs is neither mosquito-brigades nor larvicides, neither kerosene oil nor Paris green. *It is the provision of an adequate high pressure and continuous filtered and unfiltered water supply to the city.* This is no new recommendation; it was urged by James (1913), Christophers (1915), Iyengar (1920), Basu (1930), and Covell (1932). It is abundantly clear that the main breeding places of mosquitoes in Calcutta city are the reservoirs of filtered and unfiltered water. These constitute such danger as may occur of epidemic malaria from *Anopheles stephensi* breeding; of the frequent and

harassing epidemics of dengue which sweep the city; of the very great amount of suffering among the poorer class Anglo-Indians and among Hindus from filariasis. Finally, if by any chance yellow fever was introduced into the city, conditions would probably be more terrible than anything ever recorded in Panama or Central and South America.

The Act of 1876 required the Calcutta Corporation to supply filtered water continuously from 6 a.m. till 9 p.m. at 10 feet pressure, and between 7 a.m. and 9 a.m. and 5 p.m. to 6 p.m. at 50 feet pressure. The obligation on the part of the Corporation to maintain a continuous water supply was included in a Bill, which subsequently became the Act of 1899. Various engineering experts, who have been consulted on the problem, hold that a continuous water supply results in a reduction of waste. Moore's scheme—which is now partly in operation,—when completed, should yield 80 gallons per head per day, and should enable the Corporation to carry out the terms of the Act. But, as Colonel Covell remarks, 'the prospect that a continuous high pressure water supply will ever be provided appears to be remote. Hence the necessity for the storage of water in cisterns and other receptacles must continue'.

In this connection it may be of interest to compare figures for Europe and America with those for Calcutta. These are as follows :—

Average consumption of filtered water
per head per day.

American towns	..	58 to 324 gallons.
English towns	..	37 to 65 gallons.
French towns	..	31 to 58 gallons.
German towns	..	18 to 34 gallons.
Calcutta	..	51 gallons (plus 25 gallons of unfiltered water).

In the towns in Europe and America more than half the amount provided is used for trade purposes, whereas in Calcutta very little filtered water is used for trade purposes. It is obvious that any money spent on anti-mosquito measures in Calcutta city would be better spent on improvement of the water supply than on any other measure.

Even before the introduction of the filtered water supply in 1870, and of the unfiltered water supply in 1883, Calcutta city must have provided abundant suitable breeding places for mosquitoes, when wells and tanks were plentiful. In 1820 the Fever Hospital Committee reported that 'the Hindus use the Ganges water, which they collect in vessels, whilst the Europeans use rain water which they collect in Pegu jars'. In the absence of any knowledge with regard to the life-history of mosquitoes and their rôle in the transmission of tropical diseases, it is most unlikely that such receptacles were kept covered.

SUMMARY.

1. During a period of four years the density of breeding of *Anopheles stephensi* in the centre of Calcutta city has been kept under close observation in an area of one square mile in extent around the Calcutta School of Tropical Medicine. This species of mosquito pullulates in almost every receptacle for water stored throughout the city, especially in masonry tanks and overhead galvanized iron cisterns on the roofs for the filtered and unfiltered water

supplies. Out of 11,927 examinations during four years no less than 33 per cent gave positive results.

2. The correlation of the monthly incidence of *A. stephensi* breeding with the meteorological conditions in the city is shown (the latter figures being from the means of twenty years' records at Alipore). The maximum breeding occurs in July and the minimum in April.

3. During the same four years the density of breeding of *Aedes aegypti* in the same area has been under observation. The chief breeding sites are the same as those for *A. stephensi*. Out of 11,927 examinations of such sites no less than 41 per cent gave positive results. The greatest intensity of breeding was found during July and August, and the lowest in February and April.

4. The breeding of *Culex fatigans* throughout the same area was observed for two years. The chief breeding sites are the same as those of *A. stephensi* and *A. aegypti*. Out of 4,339 examinations of suspected breeding sites 8 per cent gave positive results. The greatest intensity of breeding was found in November and the lowest in July.

5. Many residents of Calcutta city acquire malaria during visits to the *mofussil*. There is continuous and heavy importation of malaria into the city by immigration from heavily endemic areas in Bengal. The local strain of *A. stephensi* can be very readily infected experimentally with malaria. Meteorological conditions for malaria transmission are suitable over a large part of the year. Yet at present malaria is but little endemic in the city. What are the reasons for this discrepancy?

6. The chief reason for the low endemicity in Calcutta appears to be that the maximum density of *A. stephensi* breeding (July-August) fails to coincide with the chief incidence of malaria cases (October-November), and especially of gametocyte carriers (December). Details are given with regard to all three species of malaria parasite, and conditions in Bombay and Calcutta are contrasted.

7. The maximum peak of *Aedes aegypti* breeding is in July and August; and this corresponds to the maximum intensity of fresh infections with dengue (August and September). Here the correlation is almost perfect. This accounts for the devastating epidemics of dengue which so often sweep the city and cause enormous financial loss.

8. New admissions for filariasis are at a fairly uniform rate throughout the year (general filaria rate 9.5 per cent). The most favourable period for transmission is during the monsoon (July-September), when the intensity of breeding of *Culex fatigans* is at a very low level. The peak for *Culex* breeding is in November, when conditions for filariasis transmission are rapidly becoming unfavourable. This want of coincidence keeps the filariasis rate at a relatively low level.

9. The cure for this state of affairs is *the provision of a continuous water supply of sufficiently high pressure to prevent mosquito breeding in the reservoirs, cisterns, etc., throughout the city*. It is the low pressure and intermittent character of this water supply which is responsible for the prevalence of mosquito-borne diseases in Calcutta.

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TABLE II.
Meteorological data for Calcutta (Alipore).

	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
Normals of maximum temperature in °F.	77.3	80.0	90.9	95.6	94.5	91.5	88.4	87.6	88.0	87.2	82.0	77.0
Normals of minimum temperature in °F.	55.5	60.0	69.3	75.7	77.5	78.8	78.6	78.4	78.0	74.3	64.3	55.0
Normals of wet-bulb temperature in °F.	59.1	62.4	70.3	76.3	78.8	80.1	79.9	79.6	79.1	75.4	66.4	58.6
Normals of dry-bulb temperature in °F.	64.8	69.3	78.5	84.2	85.8	84.9	83.6	83.0	82.6	79.8	72.0	64.4
Normals of relative humidity in per cent.	73	69	68	71	74	81	85	86	86	82	76	72
Normals of absolute humidity (vapour tension in inches of mercury).	0.433	0.481	0.642	0.808	0.899	0.966	0.974	0.969	0.949	0.825	0.582	0.422
Normals of rainfall (in inches).	0.36	1.28	1.57	1.93	5.02	12.87	13.97	13.64	9.10	4.73	0.46	0.13
Normals of wind velocity (miles per hour).	22	28	36	49	48	43	40	34	28	21	21	21
Normals of wind direction— at 8 a.m. .. at 5 p.m. ..	N. 16° W. N. 34° W.	N. 41° W. N. 67° W.	S. 56° W. S. 67° W.	S. 25° W. S. 25° W.	S. .. S. 4° W.	S. 6° E. S. 5° E.	S. 8° W. S. 4° W.	S. 3° E. S. 3° E.	S. 1° W. S. ..	N. 28° W. N. 38° W.	N. 2° W. N. 8° W.	N. 8° W. N. 14° W.

TABLE III.
Mosquito density monthly.

Monthly percentage of larvae.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
<i>Anopheles stephensi</i> (68,055) percentage of 4 years.	4.9	3.4	5.0	3.2	3.4	5.2	20.9	17.8	12.4	9.2	9.0	5.6
<i>Aedes aegypti</i> (135,489) percentage of 4 years.	5.1	3.8	4.6	3.9	4.3	4.4	17.9	18.3	14.3	8.9	8.5	6.0
<i>Culex fatigans</i> (6,104) percentage of 2 years.	11.4	6.8	4.1	9.8	10.6	8.2	2.9	7.7	6.4	5.5	15.5	11.1

TABLE IV.
Malaria, dengue and filariasis incidence monthly.

Monthly percentage of cases	January.		February.		March.		April.		May.		June.		July.		August.		September.		October.		November.		December.	
	cases	carriers	cases	carriers	cases	carriers	cases	carriers	cases	carriers	cases	carriers	cases	carriers	cases	carriers	cases	carriers	cases	carriers	cases	carriers	cases	carriers
MALARIA.—																								
<i>P. vivax</i> { cases gametocyte carriers.	32.3	18.75	47.2	34.4	55.5	50.00	36.9	52.1	36.4	22.20	52.1	68.75	36.4	22.20	42.1	0.0	36.7	20.00	30.6	8.30	30.6	16.70	27.3	5.00
<i>P. malariae</i> { cases gametocyte carriers.	24.0	18.75	11.3	18.0	8.1	0.0	21.7	8.3	10.9	0.0	21.7	6.25	10.9	0.0	8.4	80.00	4.1	10.00	2.4	0.0	4.6	11.10	10.1	0.0
<i>P. falciparum</i> { cases gametocyte carriers.	43.7	62.50	41.5	47.6	36.4	50.00	41.4	39.6	52.7	77.80	39.6	25.00	52.7	77.80	49.5	20.00	59.2	70.00	67.0	91.70	64.8	72.20	62.6	95.00
Total malaria of the year	7.7	10.7	5.3	4.9	3.9	1.3	4.6	5.1	7.5	6.0	5.1	10.7	7.5	6.0	9.5	10.0	11.1	6.7	14.9	8.0	15.2	12.0	10.3	13.3
DENGUE.—																								
Admissions for dengue cases (1,482), percentages.	1.1		0.8	1.6	1.8		3.1	9.1	12.9						20.8		19.7		18.2		7.2		3.7	
FILARIASIS.—																								
Admissions for new filariasis cases (2,796), percentages	7.1		7.5	8.1	10.3		9.6	9.2	8.8						10.0		7.7		9.1		7.9		4.7	

THE CHEMICAL COMPOSITION OF MALARIA PIGMENT (HÆMOZOIN).

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THE results of exhaustive investigations into certain properties of hæmozoin and of hæmatin have recently been published by Sinton and Ghosh (1934, 1934a) and by Ghosh and Sinton (1934). Their findings would appear to indicate that these two compounds are identical, at least in so far as the hæmozoin produced by the monkey malaria parasite, *P. knowlesi*, is concerned.

No worker seems to have determined the chemical composition of hæmozoin extracted directly from malaria parasites. Warasi (1927), however, extracted a pigment from a malarial spleen, and determined its composition after purifying it, by a complicated method of adsorption and elution from the surface of magnesium oxide. He reports the chemical composition of this substance to be carbon 51 per cent, nitrogen 13.5 per cent, hydrogen 7.6 per cent, iron 2.9 per cent and oxygen 22.5 per cent.

According to Willstätter and Fischer (1913) the formula of hæmin is $C_{33}H_{32}N_4O_4FeCl$, hence hæmatin should have the formula $C_{33}H_{32}N_4O_4FeOH$.* The percentage composition of the latter compound should, therefore, be

* There seems to be no complete agreement as to the formula of hæmatin, which is given by different authors as $C_{33}H_{32}N_4FeO_4$, $C_{33}H_{32}N_4FeO$, $C_{33}H_{32}N_4FeO$ (or O_2), $C_{33}H_{32}N_4FeO_2$, or $C_{33}H_{32}N_4FeO_3$. Hæmatin can be regarded as formed from hæmin by the replacement of Cl by (OH). We have, therefore, used the most commonly accepted formula of hæmin in making our calculation.

carbon 63.8, hydrogen 5.3, nitrogen 9.0, iron 9.0 and oxygen 13.2. The values obtained by Warasi for his malarial pigment are markedly different from those reported for hæmatin. It would appear that the substance studied by this worker was either impure, or that it was not hæmatin. It may have been, as suggested by Warasi, an iron-containing melanin. In view of these discordant results, it was decided to isolate a pure sample of hæmozoin directly from malarial parasites, and to undertake a chemical analysis of the substance. Such a procedure should eliminate all chances of the inclusion of any of the other hæmatogenous pigments which are known to occur in malarious tissue.

METHOD OF PURIFICATION OF HAEMOZOIN.

The pigment, contained in the remains of the malarial parasites of monkeys, was separated from the infected red blood cells by the method devised by Sinton and Mulligan (1932). This crude material was placed in a small porcelain basin and treated with a solution consisting of water 0.8 part, alcohol 1 part, acetic acid 1 part, and hydrochloric acid 0.2 part. The mixture was heated for about 30 minutes, the temperature being maintained a little below boiling point. More of the solution was added as it evaporated, and finally the mixture was boiled for about 6 minutes. After cooling, the residue, which was a thick viscous mass, was diluted with distilled water and centrifuged. The deposit in the centrifuge tubes consisted of crystals of pigment together with some parasitic remains, which had not yet been disintegrated completely. The deposit was washed four times with distilled water and then treated with an excess of N/20 NaOH solution. The pigment was dissolved in the alkaline solution, while the undissolved residue was removed by centrifugation.

On acidifying the alkaline extract, the pigment was precipitated. The resultant precipitate was washed several times in distilled water. It was then covered with a layer of distilled water about 3 cm. deep, and extracted repeatedly with ether mixed with alcohol and a little HCl. The pigment, which entered the ether layer, was transferred with it to a small beaker. After the whole of the pigment had been extracted, the ether solution was neutralised with NaOH and heated in a water-bath. When the ether had evaporated the pigment residue was washed thrice with distilled water, and dried in a steam chamber. The dry substance was weighed and dissolved in 2 per cent solution of quinine in chloroform, in such proportion that 50 c.c. contained 1 gm. of pigment. After about 18 hours, the solution was warmed, filtered and neutralised with HCl mixed with alcohol. On the next day it was centrifuged and the deposit washed first with alcohol three times to remove the chloroform, and then five times with distilled water. The pigment was next dissolved in an excess of NaOH solution, and the resultant solution centrifuged. The supernatant fluid was withdrawn, and acidified with a dilute solution of H_2SO_4 . The precipitate was washed four times with distilled water, re-dissolved in excess of alkali (normal NaOH), and re-precipitated with dilute H_2SO_4 . It was washed repeatedly with distilled water until free from acid, and then dried to constant weight at a temperature of $120^\circ\text{--}130^\circ\text{C}$. By this procedure only a few milligrams of purified pigment could be obtained from the blood of three monkeys showing intense infections with *P. knowlesi*.

METHODS AND RESULTS OF MICRO-CHEMICAL ANALYSIS OF PURIFIED PIGMENT.

ESTIMATION OF CARBON AND HYDROGEN.

The amounts of carbon and hydrogen were estimated by the micro-combustion method developed by Pregl (*vide* Friedrich, 1933). The results were as follows :—

Experiment I.

Weight of pigment taken	4 915 mg.
Weight of CO_2 formed	11 295 mg.
Weight of H_2O formed	2 135 mg.
Hence the carbon content found was	62 7 per cent,
and the hydrogen content	4 8 per cent.

Experiment II.

Weight of pigment taken	5 296 mg.
Weight of CO_2 formed	12 192 mg.
Weight of H_2O formed	2 410 mg.
Hence the carbon content found was	.	..	62 8 per cent,
and the hydrogen content	5 1 per cent.

ESTIMATION OF IRON

As the result of each of Experiments I and II, a residue was left which consisted of Fe_2O_3 .

Experiment I.

Weight of pigment taken	4 915 mg.
Weight of Fe_2O_3 formed	0 654 mg.
Hence the iron content found was	.	..	9 3 per cent.

Experiment II.

Weight of pigment taken	..	.	5 296 mg.
Weight of Fe_2O_3 formed	0 696 mg.
Hence the iron content found was	9 2 per cent

ESTIMATION OF NITROGEN

The nitrogen was estimated by the micro-method of Dumas, which was developed by Pregl (*vide* Friedrich, 1933).

Experiment I.

Weight of pigment taken	3 940 mg.
Volume of N. collected at 33°C. and 755 mm. pressure, over 50 per cent NaOH solution	0 223 c.c
Hence the nitrogen content found was	6.14 per cent.

DISCUSSION OF RESULTS OF ANALYSIS.

In the following table, the experimentally determined values of carbon, hydrogen, iron and nitrogen are given for the purified sample of hæmozoin obtained from monkey malarial parasites. The values of these elements, as calculated from the formula of hæmatin ($C_{33}H_{32}N_4O_4FeOH$) are also given for comparison.

TABLE.

Element	HÆMOZOIN			Hæmatin.	Warasi's pigment.
	Experiment I.	Experiment II.	Mean.		
C	62·7 per cent	62·8 per cent	62·8 per cent	63·8 per cent	51·0 per cent
H	4·8 "	5·1 "	5·0 "	5·3 "	7·6 "
N	6·1 "	6·1 "	9·0 "	13·5 "
Fe	9·3 "	9·2 per cent	9·3 "	9·0 "	2·9 "

The results obtained in our experiments are seen to differ markedly from the values recorded by Warasi (1927) in his analysis of pigment isolated from malarial tissue. His findings are also very different from the estimated composition of hæmatin.

In our results, it will be seen that the mean percentages of carbon, hydrogen and iron determined in parasitic hæmozoin, agree fairly closely with the calculated percentages of these elements in hæmatin. The estimated value of nitrogen in hæmozoin is, however, much lower than that in hæmatin. This discrepancy may be due to experimental error or to an actual difference in the chemical composition of the two compounds.

It is known that the estimation of nitrogen by the micro-method of Dumas is liable to experimental error. This is due to the formation of carbon-nitrogen compounds, which are highly resistant to combustion, and which do not give off their nitrogen freely (Friedrich, 1933), thus resulting in some instances in a low yield of this element. In such cases the use of potassium chlorate, or finely powdered potassium dichromate, along with the copper oxide, has been suggested. Unfortunately, no more purified pigment was available with which to carry out another estimation of nitrogen using these precautions. However, all the other properties of parasitic hæmozoin, so far studied, appear to agree with those of hæmatin. Furthermore, the estimated values of carbon, hydrogen, and iron contained in hæmozoin are in close agreement with the values of these elements in hæmatin. It, therefore, appears very possible that the low value for nitrogen obtained in our experiment may have been due to experimental error, and that the two pigments have the same nitrogen content. The results recorded with the other elements support the view that hæmozoin and hæmatin are identical compounds.

SUMMARY.

Hæmozoin, from blood heavily infected with *P. knowlesi*, has been purified and has been subjected to a quantitative chemical analysis by micro-methods. Its carbon, hydrogen and iron contents agree with those of hæmatin, but compared with the latter pigment the amount of nitrogen found was too low. This has been attributed to experimental error and the probable source of the error has been indicated. The results obtained with parasitic hæmozoin differ markedly from those reported by Warasi (1927) for pigment obtained from malarial tissue.

Our thanks are due to Lieut.-Colonel J. A. Sinton, M.D., D.Sc., I.M.S., Director of the Malaria Survey of India, for his valuable criticisms and encouragement. We are also indebted to Dr. K. P. Basu for his helpful advice in the course of the micro-analysis, and to Mr. P. C. Banerji for the estimation of nitrogen.

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MALARIA IN SIND.

Part XII.

A NOTE ON MALARIA IN A WATER-LOGGED AREA IN KHAIRPUR STATE.

BY

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INTRODUCTION.

PERIOD OF SURVEY

THE investigations forming the subject of this paper were carried out from 7th December to 18th December, 1933. A subsequent visit to the same area was made from 3rd February to 5th February, 1934.

TRACT SURVEYED.

The localities visited were in Khairpur and Gambat Talukas, within about 20 miles distance from the River Indus.

GENERAL DESCRIPTION OF KHAIRPUR STATE.

Khairpur State covers an area of approximately 6,000 square miles, and lies between 26° 10' and 27° 46' North Latitude and 68° 20' and 70° 14' East Longitude. It is bounded on the north-east by Sukkur District, on the east by Jodhpur and Jaisalmer States, on the south and south-west by Thar and Parkar and Hyderabad Districts, and on the north-west by the River Indus,

which separates it from Larkana and Sukkur Districts. Its greatest length is 120 miles, and its greatest breadth 70 miles.

The south-eastern half of the State forms part of the desert which occupies a large portion of Sukkur and Thar and Parkar Districts. Little cultivation is possible in this region, but it supports a scrubby vegetation which affords grazing to camels and cattle. The western and northern parts of the State are similar to the adjacent portions of Sukkur and Hyderabad Districts, and are very fertile where irrigated. A range of hills which runs south from Rohri, rising to a height of 450 feet above the sea-level and 300 feet above the surrounding plain, continues in the same general direction for about 30 miles further after passing into Khairpur State, where it spreads out to a width of 17 miles. There are no rivers in the State except the small torrents which run down from these hills after rain, and the Eastern Nara, which is now a great canal rather than a river.

The climate is that of Upper Sind generally, cold in the winter season, when severe frosts are not unknown, and very hot in the summer months, when the thermometer may rise to 120°F. The rainfall figures for the years 1928 to 1933 are given in Table I. As a rule the amount is very slight, the average for the last 13 years being 3" 59°, but in 1929 there was an exceptional precipitation of about 18 inches, almost all of which fell during the months of July and August. This was the year in which a severe regional epidemic of malaria occurred throughout northern Sind.

The population of the State, according to the census figures of 1931, was 227,183, the average density being 37 per square mile. About 80 per cent of the population are Mohammedans, and 95 per cent of these are employed in agricultural pursuits. The majority of the Hindus, on the other hand, are engaged in trade.

The staple crops are juari, bajri, wheat and cotton. Before the Lloyd Barrage Scheme came into operation in 1932, the land was irrigated by canals taking off from the River Indus, and receiving water during the inundation season only. In the cold weather some unirrigated crops were raised, particularly along the course of the Indus, and considerable cultivation of wheat, etc., was carried out by means of wells, which are particularly numerous in Khairpur and Gambat Talukas. Other products of the State are oil seeds, ghee, hides, tobacco, Fuller's earth, carbonate of soda, and wool. The manufactures comprise cotton, silken and woollen fabrics, lacquer work, carpets and pottery.

The principal inundation canal is the Mir Wah, which originally came off from the Indus just below where the Barrage is now situated. A second canal, the Abul Wah, comes off from the Indus lower down. The most important branch canals are the Faiz Wah, Faiz Baksh, Faiz Ganj, Faiz Bahar and Faiz Manj. Under the Barrage Scheme the great new Rohri Canal has been constructed, and this traverses the State from north to south, cutting across the Mir Wah about 8 miles from its head, but giving off no branches in the State itself. The upper part of the Mir Wah, from which the Faiz Wah arises, is now fed by a new canal, the Khairpur Feeder West. The lower portion is fed by another new canal, the Khairpur Feeder East. These two new canals take their origin from the Indus just above the Barrage, on either side of the Rohri Canal. Since the lower part of the Mir Wah is now completely cut off

from the upper, it follows that the upper part is now unnecessarily wide for the restricted area which it now supplies. By means of the two Feeder Canals, it is now possible to give the State regular perennial irrigation. This was actually given during the season 1933-1934, but we understand that in future the State is to receive water in the summer months only. Since the original system of inundation canals worked satisfactorily, the benefits to be received by the State from the Barrage Scheme would appear to be not very great.

The Barrage Scheme came into operation during the summer of 1932, and by August of that year there was an alarming rise in the subsoil water level in the vicinity of the Rohri Canal. An area about 20 miles long by two miles broad, with its centre opposite Khairpur Mirs, the capital of the State, rapidly became water-logged, about 7,000 acres of cultivable land being involved. Observations made by the Irrigation Department over the previous 10 years had shown that the subsoil water level in this area before the Barrage Scheme came into operation was practically constant, being about 15 to 16 feet below the surface of the ground. In this area the Rohri Canal passes through a deep belt of very fine sand, and it appears that the water-logging was caused by seepage through this of water from the canal and from the Khairpur Feeder East. It had been hoped that the bed of the canal would become sealed by the deposition of silt. But it appears that owing to the fact that the water ponded up by the Barrage during the winter is devoid of silt, this water picks up a silt charge from the canal itself, resulting in the formation of deep scour holes, and in the removal of most of the sealing material deposited during the summer. It is thus to be expected that the natural sealing of the canal will be very greatly delayed.

The total number of villages affected was 31, and many of these have disintegrated, being built entirely of mud, or of brick with mud as mortar. The towns of Khairpur and Lukman are very seriously affected, since the subsoil water level has risen under them to within three or four feet of the surface, resulting in the cracking of many buildings due to the rise of capillary water from the subsoil water table in the mud-masonry walls. To meet the emergency a number of pumps were installed, so as to keep the situation in check until more permanent remedial measures could be applied. The object of the present survey was to investigate malarial conditions in the affected area.

RESULTS OF THE SURVEY.

1. KHAIRPUR TALUKA.

Details of the spleen and blood examinations carried out in the different villages will be found in Table II.

KHAIRPUR TOWN, the headquarters of the taluka and capital of Khairpur State, is situated on the Mir Wah Canal, 15 miles east of the Indus, and 17 miles south of Rohri, and has a population of about 6,000. There are a number of tanks, fed from the Mir Wah, in and around the town, in several of which anopheline larvæ were found. The commonest species was *A. subpictus*, but *A. stephensi* was found in two of them, and *A. culicifacies* in one. The great new Rohri Canal flows within about $2\frac{1}{2}$ miles to the west of the town. Dry crops are cultivated in the surrounding country. The subsoil water level in December 1933 was from 6 to 9 feet, as measured in the wells in the town.

The spleen rate was 78 per cent (390 observations), and the average enlarged spleen measured 7.0 cm.* The parasite rate was 17 per cent (140 observations, M. T. 21, B. T. 4). Out of 313 adult anophelines captured in the town, 258 or 82 per cent were *A. stephensi*, 19 were *A. culicifacies*, and the remainder *A. pulcherrimus* or *A. subpictus*. All the specimens of *A. stephensi* and *A. culicifacies* captured were females.

NIZAMANI is a village with about 600 inhabitants, situated one mile south-west of Khairpur, and about half a mile east of Rohri Canal. There were extensive collections of seepage water close to the village, in which anopheline larvæ were found. The subsoil water level in December 1933 was 4½ feet. The spleen rate was 85 per cent (40 observations), and the average enlarged spleen measured 6.5 cm. The parasite rate was 27 per cent (40 observations, M. T. 11). Crescents were observed in one case.

LUKMAN is a village with about 3,000 inhabitants, situated about 2 miles south-west of Khairpur, and 500 yards east of the Rohri Canal. The tract between the village and the canal was flooded with seepage water, and many of the houses in the village itself were badly damaged. The soil in the flooded area was impregnated with kallar (salts), and no mosquito breeding was detected. The subsoil water level in four different wells in the village varied from 2 to 4 feet. Before the Barrage came into operation there was a certain amount of rice cultivation in the vicinity. The spleen rate in December 1933 was 96 per cent (124 observations), and the average enlarged spleen measured 5.7 cm. The parasite rate was 25 per cent (98 observations, M. T. 23, B. T. 2). Crescents were observed in two cases. Out of 85 adult anophelines captured, 64 or 75 per cent were *A. stephensi*, 7 were *A. culicifacies*, and the remainder were *A. pulcherrimus*.

RAINO is a village with a population of about 500, situated 3 miles north of Khairpur, and 1½ furlongs east of Rohri Canal. In December 1933 seepage water extended to the periphery of the village. Before the operation of the Barrage Scheme about 75 per cent of the cultivation was rice. The subsoil water level at the time of the survey was 4 feet. The spleen rate was 95 per cent (87 observations), and the average enlarged spleen measured 7.2 cm.

DAOD is a village with a population of about 400, situated 5 miles west of Khairpur, 3½ miles west of Rohri Canal, and 2½ miles west of the Faiz Wah. Between the village and the Faiz Wah there is thick forest. Dry crop cultivation extends to within half a mile of the village on three sides, whilst on the fourth it reaches its periphery. At the time of the survey in December 1933 the seepage water did not extend beyond the Faiz Wah, and the subsoil water level in the village was 14½ feet. The spleen rate was 79 per cent (28 observations), and the average enlarged spleen measured 7 cm.

MITHO MARI is a village with 150 inhabitants, situated 3 miles west of Khairpur, and 2 furlongs west of Rohri Canal. In December 1933 the village was surrounded by seepage water, which extended to the canal. Before the Barrage Scheme came into operation the chief cultivation was rice. The

* Throughout this paper the measurement of the average enlarged spleen is given in terms of the distance in centimetres from the apex of the spleen to the umbilicus.

subsoil water level at the time of the survey was 3 feet. The spleen rate was 97 per cent (39 observations), and the average enlarged spleen measured 7.9 cm.

RAMZAN PHUL POTO is a village with 225 inhabitants, situated 4 miles west of Khairpur, and 2 furlongs west of Rohri Canal, on the right bank of the Faiz Wah. A number of depressions to the east of the village contained seepage water, but no larvæ were found. Previous to the opening of the Barrage the cultivation was almost entirely rice. The subsoil water level in December 1933 was 6 to 8 feet. The spleen rate was 93 per cent (43 observations), and the average enlarged spleen measured 7.7 cm. Out of 59 adult anophelines captured, 55 or 93 per cent were *A. stephensi*, the remainder being *A. subpictus*.

KHANPUR is a village with a population of about 500, situated 4 miles west of Khairpur, and a mile west of Rohri Canal, on the right bank of the Faiz Wah. In December 1933 the area between the Faiz Wah and Rohri Canal was flooded with seepage water, in which anophelines were breeding freely. There was rice cultivation at a distance of about a mile to the west of the village. The subsoil water level was 2 to 6 feet. The spleen rate was 91 per cent (104 observations), and the average enlarged spleen was 6.6 cm. Out of 38 anophelines captured, 34 or 89 per cent were *A. stephensi*, the remainder being *A. pulcherrimus*.

TERHI is a village with a population of about 1,500, situated 4 miles north of Khairpur, and half a mile west of the Rohri Canal. To the west of the village there is a large pond in which anopheline larvæ were found. Before the Barrage came into operation the chief cultivation was rice. The subsoil water level in December 1933 was between 4 and 7 feet. The spleen rate was 89 per cent (105 observations), and the average enlarged spleen measured 6.8 cm.

BABARLO is a village with 1,500 inhabitants, situated 10 miles north of Khairpur, and one mile west of Rohri Canal. This village was not affected by seepage from the canal. A distributary from the Abul Wah flows within 150 yards of its edge. In December 1933 the subsoil water level was 11 feet. There was no rice cultivation in the vicinity. The spleen rate was 93 per cent (71 observations), and the average enlarged spleen measured 8.4 cm.

TANDO MASTIKHAN is a village with 1,500 inhabitants, situated 10 miles south of Khairpur, and 2½ furlongs west of Rohri Canal. The Faiz Wah flows about 2 furlongs west of the village. In December 1933 the seepage water extended to within about one furlong from the village, and numerous larvæ of *A. stephensi* were found in it. There were also two large water collections close to the village. Previous to 1932 there was a small amount of rice cultivation in the vicinity. The subsoil water level at the time of the survey was 6½ feet, and the villagers stated that there had been a rise in its level of about 6 feet since the Barrage Scheme had come into operation. The spleen rate was 90 per cent (200 observations), and the average enlarged spleen measured 6.8 cm. The spleen rate among adults was 60 per cent (40 observations). The parasite rate was 41 per cent (63 observations, M. T. 26). Out of 107 adult anophelines captured in the village, 89 or 83

per cent were *A. stephensi*, and 9 were *A. culicifacies*. The remainder were *A. subpictus* and *A. pulcherrimus*.

BAHARO KHAN LASHARI is a village with about 100 inhabitants, situated 8 miles south of Khairpur, and $\frac{1}{4}$ mile east of the Rohri Canal. There was no water-logging in the vicinity, and the subsoil water level was 12 feet, but the inhabitants stated that it was more than 30 feet before the Barrage Scheme came into operation. There was no rice cultivation in the vicinity. The spleen rate was 62 per cent (21 observations), and the average enlarged spleen measured 7.9 cm. Out of 19 adult anophelines captured, 16 or 84 per cent were *A. stephensi*, two *A. culicifacies* and one *A. subpictus*.

BURDI is a village with about 100 inhabitants, situated 7 miles south of Khairpur, and $1\frac{1}{2}$ miles east of Rohri Canal. There was no seepage water within one mile of the village in December 1933, and the subsoil water level was 15 feet. There was no rice cultivation in the vicinity. The spleen rate was 66 per cent (6 observations only). Out of 17 adult anophelines captured in the village, 11 or 65 per cent were *A. stephensi*, and the rest *A. subpictus*.

UMAID ALI LASHARI is a village with 150 inhabitants, situated 8 miles south of Khairpur, and $1\frac{1}{2}$ miles east of Rohri Canal. There was no water-logging in the vicinity, and the subsoil water level was 14 feet. The villagers said that this had risen by about 4 feet since the Barrage Scheme began to operate. There was no rice cultivation nearby. The spleen rate was 25 per cent (24 observations), and the average enlarged spleen measured 8.3 cm. Out of 7 adults examined, none was found with enlarged spleen. Three out of five adult anophelines captured were *A. stephensi*.

BAKU KHAN LASHARI is a village with about 100 inhabitants, situated 8 miles south of Khairpur, and half a mile east of Rohri Canal. Seepage water extended to within about quarter of a mile from the village in December 1933. The subsoil water level was 12 feet, and the villagers said that it had risen by about 5 feet since the Barrage Scheme began to operate. The spleen rate was 37 per cent (24 observations), and the average enlarged spleen measured 8.1 cm. Out of 13 adults examined, one was found to have an enlarged spleen.

KANASARA, a village with about 150 inhabitants, was situated $3\frac{1}{2}$ miles south of Khairpur on the right bank of the Rohri Canal, but, as the original village was completely destroyed by the seepage from the canal, the people were housed in temporary huts on the left bank at the time of the survey. There was some rice cultivation near the village before the Barrage came into operation. The subsoil water level in December 1933 was 2 feet, but the villagers said it had been about 25 feet before the Barrage Scheme began to operate. Larvæ of *A. stephensi* were found in the seepage water, and 9 out of 16 adults captured in the village were of this species. The remainder were either *A. subpictus* or *A. pulcherrimus*. The spleen rate was 77 per cent (13 observations only), and the average enlarged spleen measured 6.0 cm. Seven out of 11 adults examined had enlarged spleens.

SOHOO is a village with 600 inhabitants, situated 6 miles south of Khairpur, and 200 yards east of Rohri Canal. The tract between the canal

and the village was flooded with seepage water, in which the larvæ of *A. stephensi* were found. There was a little rice cultivation near the village before the Barrage Scheme came into operation. The subsoil water level in December 1933 was $3\frac{1}{2}$ feet, but the villagers stated that before the opening of the Barrage it was about 25 feet. The spleen rate was 78 per cent (51 observations), and the average enlarged spleen measured 7.4 cm. The parasite rate was 60 per cent (20 observations, M. T. 12). Crescents were observed in two cases. Five out of 16 adults examined had enlarged spleens.

PANERO is a hamlet with about 50 inhabitants, situated 8 miles south of Khairpur, and 200 yards east of Rohri Canal, and surrounded by dry crop cultivation. Before the Barrage came into operation there was a small amount of rice grown also. The subsoil water level in a well situated 250 yards from Rohri Canal was 3 feet, but the villagers said it had been 25 feet before the opening of the Barrage. The spleen rate was 61 per cent (23 observations), and the average enlarged spleen was 8.5 cm. Out of 29 adult anophelines captured, 21 or 72 per cent were *A. stephensi*, and the rest were *A. culicifacies*.

2. GAMBAT TALUKA.

The results of spleen and blood examinations made in villages in this taluka are given in Table III.

FATEHPUR is a village with about 500 inhabitants, situated 6 miles north-west of Gambat, and 200 yards east of the Rohri Canal. A branch canal from the original irrigation system runs within 50 yards of the village. Anopheline larvæ were found in a number of excavations in and around the village. The subsoil water level in a well near the village was $4\frac{1}{2}$ feet, but in the village itself it varied between 12 and 15 feet, because the site is elevated somewhat above the surrounding country. The spleen rate was 81 per cent (47 observations), and the average enlarged spleen measured 7.1 cm. Out of 32 adult anophelines captured, 23 or 74 per cent were *A. stephensi* and the rest *A. culicifacies*.

SHAH JO TAKYA is a village with about 60 inhabitants, situated in the midst of dry crop cultivation, $1\frac{1}{2}$ miles east of Gambat, and 50 yards east of Rohri Canal. There was no well in the village, so that the level of the subsoil water could not be measured. The spleen rate was 72 per cent (18 observations), and the average enlarged spleen was 6.9 cm.

SHADI PHUL is a village with about 100 inhabitants, situated 4 miles west of Gambat, on the right bank of Rohri Canal. There was no rice cultivation in the vicinity. The subsoil water level in December 1933 was 23 feet. The spleen rate was 54 per cent (11 observations only), and the average enlarged spleen measured 7.8 cm.

KOHORA is a village with 2,600 inhabitants, situated 3 miles north-west of Gambat, and 2 miles west of Rohri Canal. The Abul Wah flows within 25 yards of the western edge of the village. There is no rice cultivation in the vicinity. The villagers stated that in 1929 the floods extended to the edge of the village. In December 1933 the subsoil water level varied from 18 to 25 feet. The spleen rate was 68 (168 observations), and the average enlarged spleen measured 7.6 cm. The parasite rate was 22 per cent (100 observations,

M. T. 22). Crescents were observed in 8 cases. Out of 22 adult anophelines captured, 5 were *A. stephensi*, 13 *A. culicifacies*, and the rest *A. subpictus*.

GUJHRA is a village with about 150 inhabitants, situated 1 mile south-east of Gambat, and 50 yards west of Rohri Canal, in the midst of dry crop cultivation. In December 1933 the subsoil water level was 24 feet, and the villagers stated that it had risen by about 4 feet since the Barrage Scheme came into operation. The spleen rate was 54 per cent (28 observations), and the average enlarged spleen measured 8.2 cm.

GEHALPUR is a village with about 150 inhabitants, situated one mile south of Gambat, and 25 yards west of Rohri Canal, surrounded by dry crop cultivation. The subsoil water level in December 1933 was 24 feet, and the villagers said it had been about 35 feet before the Barrage commenced to operate. The spleen rate was 65 per cent (26 observations), and the average enlarged spleen was 8.2 cm.

GAMBAT is a small rural town, with a population of about 5,000, situated 20 miles south-west of Khairpur, and half a mile west of Rohri Canal. Two distributaries from the Abul Wah flow within 400 yards of the town. There is no rice cultivation in the vicinity. The subsoil water level in December 1933 was 25 to 30 feet. The spleen rate was 59 per cent (337 observations), and the average enlarged spleen measured 7.4 cm. The parasite rate was 36 per cent (120 observations, M. T. 42, B. T. 1). Crescents were observed in 9 cases. Out of 16 adult anophelines captured, 15 were *A. culicifacies*, and one *A. stephensi*.

ANOPHELINE MOSQUITOES.

The numbers of the various species of anophelines captured as adults during the course of the survey were as follows:—

Species.	Males.	Females.	TOTAL.
<i>A. stephensi</i> ..	45	544	589
<i>A. culicifacies</i> ..	2	84	86
<i>A. subpictus</i> ..	2	49	51
<i>A. pulcherrimus</i>	31	31

The striking feature with regard to these figures is the very marked preponderance of *A. stephensi*, which formed 87 per cent of the catch in the water-logged area. No such result has been met with by us in any other locality in Sind during the course of observations extending over 7 years. It is true that a few examples of this species have occasionally been caught in the course of our surveys, but never in numbers approaching those of the three common species of Sind, namely *A. culicifacies*, *A. subpictus* and *A. pulcherrimus*.

The chief breeding places of *A. stephensi* were collections of seepage water in the proximity of Rohri Canal, and in certain tanks. Larvæ of *A. culicifacies* were chiefly found in small canal distributaries and in collections of water in close proximity to these. Those of *A. subpictus* were found in collections of

dirty water in and about the villages, whilst *A. pulcherrimus* was found breeding in various ponds and swamps.

Out of 250 specimens of *A. stephensi* collected in Khairpur Town in December 1933, 6, or 2.4 per cent were found to have gut infections, masses of sporozoites being seen in one instance. No infections of salivary glands were met with.

DISCUSSION.

Out of the 25 villages in which observations were carried out, 14 were completely water-logged, whilst the remaining 11 were situated in areas which were not water-logged, though the subsoil water level had risen by several feet in most cases. The results of spleen examinations carried out in the villages are given separately in Tables IV and V. The combined spleen rate in the 14 water-logged villages (1,294 observations) was 86 per cent, and the apex-umbilicus measurement of the average enlarged spleen was 6.9 cm. In the remaining 11 villages the combined spleen rate was 63 per cent (734 observations), and the average enlarged spleen measurement was 7.8 cm.

As regards the blood examinations (Tables VI and VII), out of 361 children examined in the water-logged area, 27 per cent were found infected, with an average parasite value of 974 per c.mm. of blood. Crescents were observed in 5.4 per cent of the malignant tertian cases, which formed 9.4 per cent of the total. In the non-water-logged area, out of 220 children examined 29.5 per cent were found infected, with an average parasite value of 1,700 per c.mm. of blood. Crescents were observed in 26 per cent of the malignant tertian infections, which constituted 98 per cent of the total.

Thus in the water-logged area the spleen rate was higher and the average enlarged spleen greater than in the non-water-logged area. The parasite rate was approximately the same in each case, but in the non-water-logged area the higher average parasite value and the greater proportion of crescent carriers suggests that there were a larger number of acute infections, associated with a lesser degree of communal immunity.

The morbidity rates, as portrayed in the hospital figures of Khairpur and the dispensary figures of Gambat for the last 6 years, are given in Tables VIII and IX. In both cases the effects of the regional epidemic of 1929, which affected the whole of northern Sind, are clearly shown. In the two following years the morbidity rate during the autumn months fell considerably, reaching what is presumably its normal figure in 1931; but in 1932 and 1933 there was a marked increase in the October and November figures. This is in accordance with our findings in Larkana District during these years, where we attributed the increase of malaria to the effects of the operation of the Lloyd Barrage Scheme (Covell and Baily, 1934).

The question arises as to how far the high degree of malaria now existing in the water-logged area of Khairpur State may justly be attributed to the water-logging itself, i.e., to the rise of subsoil caused by seepage from the bed of the Rohri Canal. It is unfortunate that no observations regarding the incidence of malaria were made before December 1933, but certain conclusions may be drawn from the data at our disposal. It was ascertained that prior to the opening of the Lloyd Barrage there was a considerable amount of rice

cultivation in the vicinity of the villages which subsequently became water-logged. Our experience in other parts of Sind has shown that in such localities there is normally a high incidence of malaria, and it is probable that these villages would always show a fairly high spleen rate.

The morbidity figures for Khairpur and Gambat show that the area was severely affected by the 1929 epidemic, but under normal circumstances we would have expected the incidence of malaria to have progressively decreased during the succeeding years. Instead of this we have evidence of a decided increase in the years 1932 and 1933, i.e., during the first two years of the operation of the Barrage. There is no doubt that the conditions produced by the seepage from the Rohri Canal have played a great part in the production of the very high malaria incidence in the water-logged area.

Not only has there been a rise in subsoil water level due to seepage from the Rohri Canal, but also the existing canals of the State, which were formerly filled only in the inundation season, have received water since 1932 throughout the year. Whether the prevalence of *A. stephensi*, a dangerous malaria carrier, in the area is due to the altered conditions cannot be said with certainty. But since a preponderance of this species has never before been encountered by us in Sind, it seems possible that this species may have found the widespread water-logging of the country especially favourable to its needs.

As to the remedy for the present state of affairs, this lies in the hands of the irrigation engineers, who are confronted with a very difficult task. The various schemes which have been proposed to deal with the situation are briefly as follows :—

(1) To leave things as they are till the canal is naturally sealed up, the damaged land being acquired by Government.

(2) As in (1), but with the addition of a drainage scheme, and the installation of pumps to allow the land to be cultivated.

(3) As in (1), but to construct two parallel drainage trenches, one on either side of the canal, with the object of cutting off as much as possible of the seepage water now passing into the subsoil. Water would have to be removed from the trenches by pumps.

(4) To accelerate the rate of sealing by pumping in fine material at the head of the canal.

(5) To try and prevent scour, (a) by widening the canal and so reducing the velocity of the current, or (b) by covering the worst places by mattresses of wire netting and brushwood.

(6) To deepen the canal by 8 feet, by constructing a new fall near the head of the canal, instead of at Tando Mastikhan, where it is at present.

(7) To line the canal with cement concrete, or some other non-porous material.

(8) To construct a power house at Tando Mastikhan, using the fall as the source of power, and to distribute electrical energy from this to tube wells.

(9) To seal off the canal by injecting cement, clay or some sort of chemical into the sand below the bed.

Propositions (6) and (7) would involve the closing of the canal for several months, so that the loss from the winter crop throughout the whole area supplied by the canal would be added to the expense. Experiments are being

carried out to find the most suitable remedy, but it seems probable that the proposition first laid down above, with the addition of some sort of drainage scheme, will be adopted.

Apart from engineering projects designed to deal with the water-logging problem, anti-mosquito measures are out of the question, and the only step to be recommended is the provision of adequate treatment for the sick.

SUMMARY,

1. As the result of seepage from the great new Rohri Canal and the Khairpur Feeder East, a considerable area in Khairpur State, including over 7,000 acres of cultivable land and 31 villages, became completely water-logged, almost immediately after the opening of the Lloyd Barrage in 1932.

2. A malaria survey carried out in December 1933 showed that the combined spleen rate in 14 of the villages which were completely water-logged was 86 per cent. In 11 villages which were not water-logged, but where the subsoil water level had risen by several feet, the combined spleen rate was 63 per cent.

3. The whole area was severely affected by the regional malaria epidemic of 1929. It is probable that there is normally a fairly high incidence of malaria in this tract. We consider, however, that the great rise in the subsoil water level following the opening of the Barrage has been the direct cause of the increased incidence of malaria in 1932 and 1933.

4. A remarkable feature of the survey was the great preponderance of *A. stephensi* over the other species of anophelines captured. In the course of 7 years' observations in various parts of Sind this species has never before been met with, except in very scanty numbers.

5. There seems to be no immediate prospect that the water-logging of this area will be abolished in the near future. This being so, we may expect the incidence of malaria to continue to be high. Apart from engineering operations to reduce the water-logging, there can be no question of employing effective anti-mosquito measures, and the only procedure recommended is the provision of adequate facilities for the treatment of the sick.

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APPENDIX.

TABLE I.

Monthly amounts of rainfall recorded in inches at Khairpur, 1928-33.

Months.	1928.	1929.	1930.	1931.	1932.	1933.
January	0'15	0'40	0'10
February	0'16
March
April ..	0'27	0'13
May	0'10
June	0'30	0'83	0'75	..
July ..	0'45	1'18	2'13	..	2'61	0'40
August	6'59	0'84	3'97
September
October	0'70
November ..	0'50
December
TOTAL ..	1'24	18'39	2'47	1'60	4'20	4'64

TABLE II.

Results of spleen and blood examinations in Khairpur taluka.

Locality.	SPLEEN RESULTS.				BLOOD RESULTS.		
	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*	Number examined.	Number with parasites.	Parasite rate.
Khairpur ..	390	305	78'2	7'0	140	24	17'1
Nizamani ..	40	34	85'0	6'5	40	11	27'5
Lukman ..	124	120	96'6	5'7	98	25	25'5
Raino ..	87	83	95'4	7'2
Daod ..	28	22	78'6	7'0
Mitho Mari ..	39	38	97'4	7'9
Ramzan Phul Poto ..	43	40	93'0	7'7
Khanpur ..	104	95	91'3	6'6
Terhi ..	105	94	89'5	6'8
Babarlo ..	71	66	93'0	8'4
Tando Mastikhan ..	200	180	90'0	6'8	63	26	41'3
Baharo Khan Lashari ..	21	13	61'9	7'9
Burdi ..	6	4	66'7	9'1
Umaid Ali Lashari ..	24	6	25'0	8'3
Baku Khan Lashari ..	24	9	37'5	8'1
Kanasara ..	13	10	76'9	6'0
Sohoo ..	51	40	78'4	7'4	20	12	60'0
Panero ..	23	14	60'9	8'5
TOTAL ..	1,393	1,173	84'2	6'9	361	98	27'1

* Measurement in centimetres from the apex of the spleen to the umbilicus.

TABLE III.

Results of spleen and blood examinations in Gambat taluka.

Locality.	SPLEEN RESULTS.				BLOOD RESULTS.		
	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*	Number examined.	Number with parasites.	Parasite rate.
Fatehpur	47	38	80.9	7.1
Shah Jo Tukya ..	18	13	72.2	6.9
Shadi Phul	11	6	54.5	7.8
Kohora	168	114	67.9	7.6	100	22	22.0
Gujhra	28	15	53.6	8.2
Gehalpur	26	17	65.4	8.2
Gambat	337	200	59.3	7.4	120	43	35.8
TOTAL	635	403	63.5	7.6	220	65	29.5

* Measurement in centimetres from the apex of the spleen to the umbilicus.

TABLE IV.

Results of spleen examinations in water-logged villages.

Locality.	Number examined.	Number with enlarged spleen	Spleen rate	Average enlarged spleen.*
Khairpur	390	305	78.2	7.0
Nizamani	40	34	85.0	6.5
Lukman	124	120	96.6	5.7
Raino	87	83	95.4	7.2
Daod	28	22	78.6	7.0
Mitho Mari	39	38	97.4	7.9
Ramzan Phul Poto ..	43	40	93.0	7.7
Khanpur	104	95	91.3	6.6
Terhi	105	94	89.5	6.8
Tando Mastikhan ..	200	180	90.5	6.8
Kanasura	13	10	76.9	6.0
Sohoo	51	40	78.4	7.4
Panero	23	14	60.9	8.5
Fatehpur	47	38	80.9	7.1
TOTAL	1,294	1,113	86.0	6.9

* Measurement in centimetres from the apex of the spleen to the umbilicus.

TABLE V.

Results of spleen examinations in non-water-logged villages.

Locality.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
Babarlo	71	66	93'0	8'4
Burdi	6	4	66'7	9'1
Umaid Ali Lashari	24	6	25'0	8'3
Baku Khan Lashari	24	9	37'5	8'1
Shah Jo Takya	18	13	72'2	6'9
Shadi Phul	11	6	54'5	7'8
Kohora	168	114	67'9	7'6
Gujhra	28	15	53'6	8'2
Gehalpur	26	17	65'4	8'2
Gambat	337	200	59'3	7'4
Baharo Khan Lashari	21	13	61'9	7'9
TOTAL ..	734	463	63'0	7'8

* Measurement in centimetres from the apex of the spleen to the umbilicus.

TABLE VI.

Results of blood examinations in water-logged villages.

Locality.	Number examined.	Number with parasites.	Parasite rate.	Average positive parasite value *	Number with M. T. infections.	Number with B. T. infections.	Number with crescents.
Khairpur ..	140	24	17'1	482	21	4	
Nizamani ..	40	11	27'5	585	11	..	1
Lukman ..	98	25	25'5	968	23	2	2
Tando Mastikhan ..	63	26	41'3	1,263	26	..	
Sohoo ..	20	12	60'0	1,705	12	..	2
TOTAL ..	361	98	27'0	974	93	6	5

TABLE VII.

Results of blood examinations in non-water-logged villages.

Kohora ..	100	22	22'0	761	22	..	8
Gambat ..	120	43	35'8	2,181	42	1	9
TOTAL ..	220	65	29'5	1,700	64	1	17

* Per c.mm. of blood.

TABLE VIII.

Number of out-door cases treated monthly at the Civil Hospital Khairpur during the years 1928 to 1933.

Months.	1928.	1929.	1930	1931.	1932.	1933.
January ..	3,160	3,141	4,272	4,464	3,769	4,573
February ..	2,610	2,616	4,167	3,059	3,707	3,787
March ..	2,874	2,667	3,423	2,137	4,127	4,167
April ..	3,083	2,741	3,089	2,966	3,421	3,743
May ..	2,930	2,727	2,758	2,366	3,263	3,726
June ..	2,407	2,240	2,269	2,936	2,642	3,359
July ..	2,448	3,263	2,482	2,929	1,798	3,833
August ..	2,371	3,785	2,938	2,892	3,027	4,056
September ..	2,598	5,688	3,512	3,191	3,685	3,696
October ..	3,283	9,407	4,459	3,883	6,594	9,738
November ..	3,628	6,679	4,218	3,957	7,162	5,535
December ..	3,868	9,928	4,114	3,564	6,549	..

TABLE IX.

Number of cases treated monthly at Gambat dispensary during the year 1928 to 1933.

Months.	1928.	1929.	1930	1931	1932.	1933.
January ..	1,533	1,575	2,586	1,635	1,973	1,713
February ..	1,319	1,367	2,212	1,584	2,397	1,647
March ..	1,772	1,774	2,232	1,850	2,465	1,934
April ..	1,712	1,746	2,185	1,633	1,989	1,975
May ..	1,170	1,379	1,984	1,438	1,786	1,924
June ..	1,331	1,297	1,607	1,446	1,534	1,536
July ..	1,558	1,261	1,866	1,479	1,705	1,806
August ..	1,775	1,502	2,256	1,680	2,043	2,111
September ..	2,007	3,466	2,098	2,014	2,568	2,755
October ..	3,023	6,041	2,697	3,530	3,208	5,158
November ..	2,470	4,593	2,451	3,115	3,139	4,252
December ..	1,834	3,115	1,864	1,998	2,181	..

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	Spectra Studies in Malarial Sera	KEHAR.

(Continued on opposite page of Cover).

ANTI-MALARIAL OPERATIONS IN THE VIZAGAPATAM HARBOUR CONSTRUCTION AREA (1927—1933).*

BY

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[16th July, 1934.]

I. INTRODUCTION.†

Vizagapatam town is situated on the coast of the Bay of Bengal (longitude 83°3'E. and latitude 17°75'W.). It has a land area of 6·13 square miles, with a population of 57,303 according to the census of 1931. Maps I and II show details of the topography of this area.

Vizagapatam harbour.—This port will serve the north-eastern portion of the Madras Presidency, the southern parts of Orissa, and also to some extent the Central Provinces to which it is connected by a railway line.

The commercial importance of this port is expected to grow very much in the future. Side by side with this, the activity of the Public Health Department will also inevitably increase, especially in view of the new railway connection between Raipur in the Central Provinces and Vizagapatam, not to speak of sea and air communications.

Climate.—The rains usually begin in the month of June and end in November. The average annual rainfall is about 40 inches. The south-west monsoon begins in June and the north-east in October. The highest maximum relative humidity was 100 per cent recorded in July 1932, while the lowest was 39 per cent reported in February 1930. The prevailing winds blow from the south-west for about 8 months of the year.

* This report has been very largely condensed and re-arranged. The original report, which has been preserved for reference in the library of the Malaria Survey of India, contained many additional charts, tables and photos, which it has not been possible to publish.—(*Editor.*)

† A foreword to this report has been written by O. B. Rattenbury, Esq., the Engineer-in-Chief, Vizagapatam Harbour Construction (*vide* Appendix I).

The variations between the extremes of temperature are very slight, the average maximum and minimum temperatures being 87°F. and 75°F. respectively. During the past 3 years the highest maximum temperature recorded was 104°F. in June 1931, while the lowest minimum was 61°F. in December 1930, 1931 and 1932, and January 1931 and 1932.

II. HISTORY OF MALARIAL SURVEYS.

It was Major Perry, I.M.S., in 1911 who first pointed out that the intensity of malaria on the southern shore of the Harbour was greater than in the town of Vizagapatam (Perry, 1914). In 1913 Major Ross, I.M.S., and in 1925 Dr. K. V. Krishnan (1929) made short malaria surveys of this area. After the Harbour construction had commenced Mr. Senior-White made two detailed surveys, one in March 1926, and the other in the following November. His investigations again showed that there was a high malarial prevalence in the villages on the south shore, namely Tepparevupalam, Chintalapalam, Valakampeta and Malakapuram. As a result of his work he drew up some tentative proposals for the protection of various sites in the vicinity of the Harbour. Briefly these suggestions were :—

(i) The removal from the south shore of the malarious villages mentioned above.

(ii) The improvement of drainage; the training of the geddas* down to half-tidal level; stone-pitching of the geddas within a quarter mile radius of inhabited areas, and the construction of hill-foot contour drains and roadside drains.

He suggested the appointment of a trained malariologist to keep a constant supervision during the construction work. He also recommended the employment of a European Sanitary Inspector to carry out temporary anti-mosquito measures, such as oiling, etc.

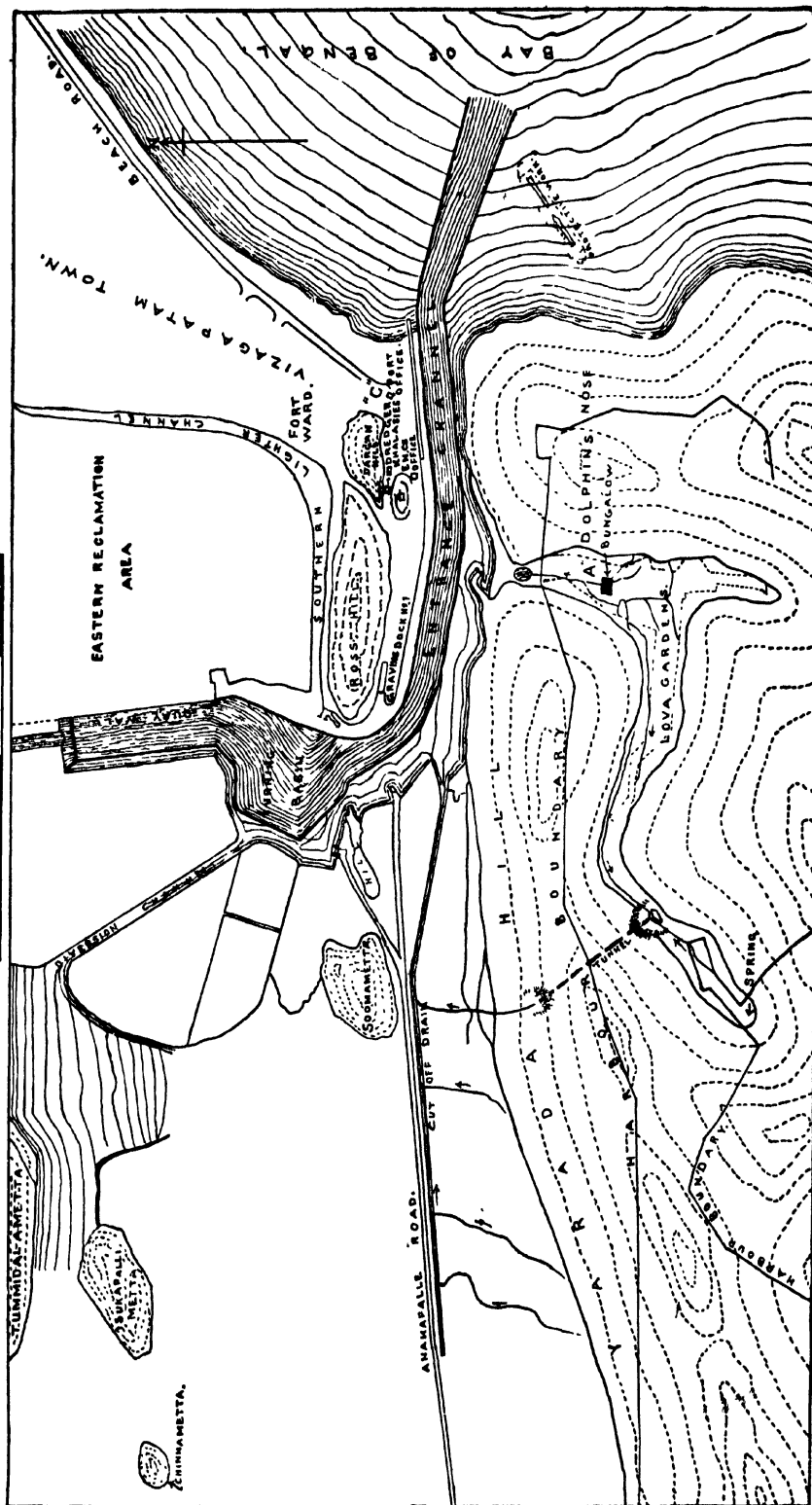
In his second report, Senior-White records the presence in the Lova Gardens of the dangerous malaria-carrying anophelines, *A. funestus*†, *A. culicifacies*, *A. jeyporiensis* and *A. theobaldi*. He suggested that the swamp in the lower portions of this valley should be filled in, as he considered it responsible for the breeding of these species. He found *A. funestus*† breeding in every gedda on the Malakapuram side of the south shore near the malarious villages mentioned. He was, however, unable to confirm the presence of *A. stephensi* in these geddass, as recorded by Krishnan, but agreed in the necessity of closing the wells in this locality.

In December 1926 Lieut.-Colonel Fraser, I.M.S., then Chief Medical Officer of the Harbour, had actually recognised the necessity for anti-malarial operations. He also pointed out to the Engineering Authorities that isolated pools along the embankment opposite the workshops were breeding anophelines, and considered it imperative that the breeding should be abolished by giving these pools a free tidal communication.

* Geddass are small hill streams.

† Under the name '*A. funestus*' Senior-White included *A. fluviatilis* (*A. listoni*), *A. aconitus* and *A. minimus*.—(Editor.)

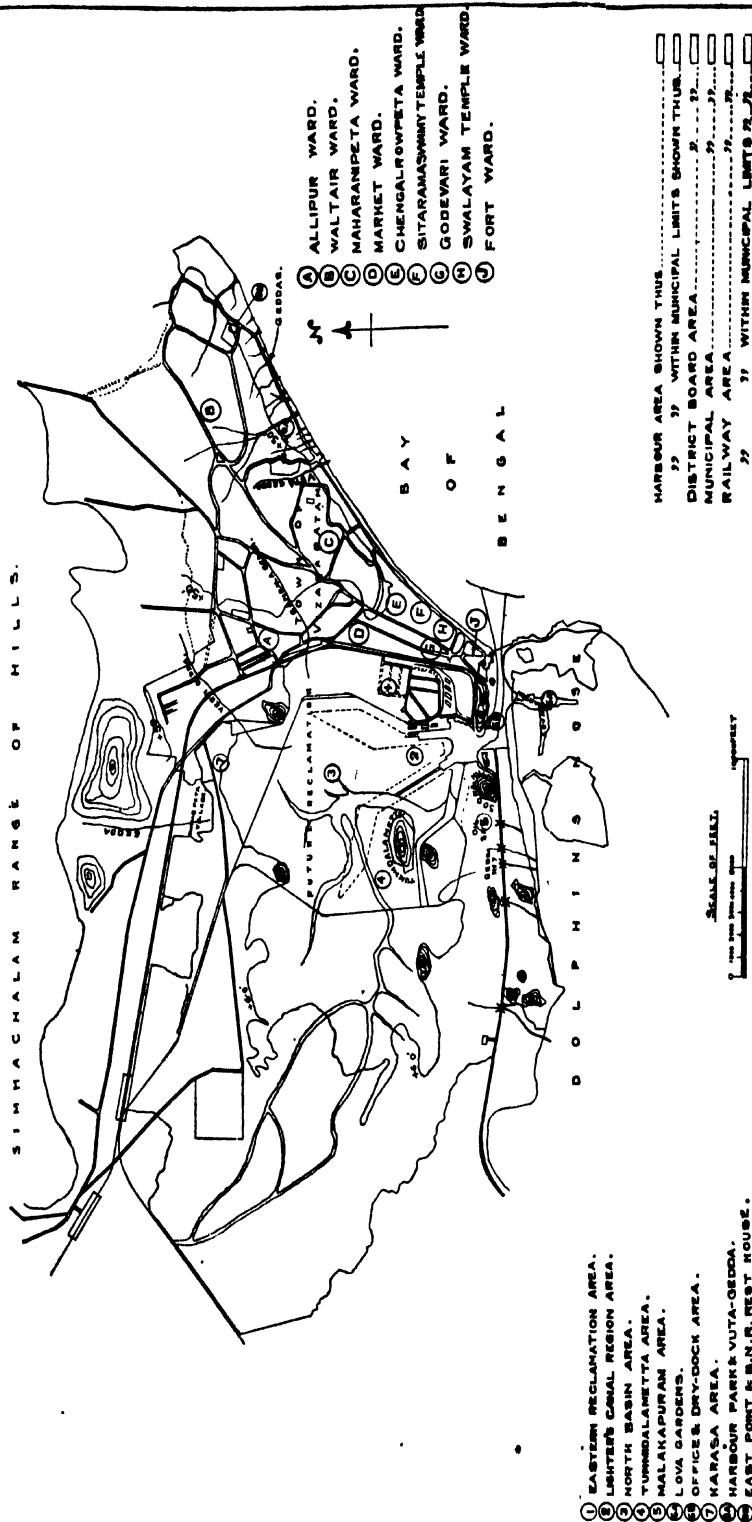
SOUTHERN SIDE OF HARBOUR



REFERENCE:-

MAP II.

VIZAGAPATAM HARBOUR AND SURROUNDINGS.



In March and April 1927 Dr. Venkataraman (1929a, 1929b) made a survey of Vizagapatam proper. He found that it was not very malarious, as the average spleen rate was below 10 per cent.

A. stephensi in the denser parts of the town and *A. culicifacies* in the outlying areas were the two species 'most suspected of carrying malaria'. Out of 40 wells examined by this worker in the Chengalrowpeta, Fort and Temple wards of the town, 20 were found to be breeding *A. stephensi*.

The investigations of Venkataraman were continued by Dr. Raghavendrarao in October 1927. The latter noted that the incidence of malaria was not so great within the heart of the town as in the neighbourhood of the geddas. He, therefore, thought that the malaria problem was intimately associated with these streams and with the swampy area. The commonest malaria vector found by him in different parts of the town was *A. culicifacies*. *A. stephensi* came next in numbers, and was breeding both in the geddas and in wells. During a visit to the Lova Gardens he was only able to collect 5 anopheline larvæ after a prolonged search, and two adults hatching from these proved to be *A. tessellatus*.

Raghavendrarao recommended the straightening, training and canalisation of the Yerri, the Gangulu and the Voota geddas, which were breeding *A. culicifacies*. He also suggested the closure or filling up of disused wells, and the stocking of wells in daily use with larvicidal fish.

At this time the Harbour Authorities were reclaiming much swampy ground in their area by hydraulic filling. Raghavendrarao, therefore, suggested that the Municipal Authorities might negotiate with them to fill up the swamp inside Municipal limits.

During the months of February 1926 and February 1927, there was an increased prevalence of mosquitoes. This plague was wrongly attributed to the effect of the Harbour construction work, which had then begun to enclose by embankments areas along the seashore. The Harbour Authorities had, however, taken great care to see that borrow-pits dug during this period were subject to tidal flooding to prevent mosquito breeding. Oiling had also been started in the immediate vicinity of the Harbour, in the Lova Gardens, and, from January 1927, along the nearest half mile of the south shore of the Harbour. At this time no anti-malarial operations had yet been commenced either in the town of Vizagapatam or in the B. N. Railway area.

Later, in November 1927, Lieut.-Colonel J. A. Sinton, I.M.S., visited Vizagapatam in connection with the proposed anti-malarial operations. He approved of the scheme proposed by Senior-White in its main details. He advised the use of subsoil drainage in certain areas, and that the sides of the geddas should be stone-pitched, while the bottom of these streams should have a central cement drain to carry off the permanent flow. He considered that in many places during the construction operations it would be necessary to be dependent upon oiling.

Sinton also advised filling and drainage in several areas. He recommended that at first no permanent masonry drains should be made in the Lova Gardens, but that a temporary main central earth drain with appropriate lateral drainage should be constructed in this valley. When the diversion of the spring in the

upper part of the ravine had been completed, and the effect of temporary drains observed, it would then be possible to consider the alignment and character of the more permanent drainage to be installed in this area.

This worker approved of the suggestion that a large 'pucca', 'cut-off' or intercepting drain should be constructed along side the Anakapalli Road. This drain would intercept the water coming in the geddas from the hills on the south shore before it could enter the large seepage area. It was expected that this drain would reduce the area of swampy breeding ground along the shore, and, when it had acted for some time, would indicate those places in which permanent subsoil drainage would be needed to supplement its action.

An area south of the line of this channel, nearly opposite Culvert No. 4, was considered by him to be the most suitable site for a labour camp on the south shore.

Sinton thought that the three species of Anophelines concerned in the carriage of malaria in this area were *funiatis* (*listoni*), *culicifacies* and *stephensi*.

The Malaria Commission of the League of Nations visited this locality in 1929 and remarked as follows :—

'The work, which shows its good results by the declining number of malaria cases treated at the Hospital of the Port, is of particular interest, because it has been undertaken, not as a consequence of a serious outbreak of malaria, but to prevent such an outbreak amongst the present labour force and especially among the personnel of the port after it has been opened'.

The chief malarial surveys by different workers were completed in 1927. The Harbour Authorities had, however, already started temporary anti-mosquito measures, such as oiling, etc., as early as January of that year. An intensive campaign was carried out from the latter part of 1927 until March 1930 by Mr. J. A. Donald, an Assistant Engineer of the Harbour, under the supervision of Lieut.-Colonel Fraser, I.M.S.

The author took over charge of the work in May 1930, and the result of his work is recorded below. These observations may be of interest to other malariologists engaged in similar work. It will, however, be necessary to continue the study for some years before any definite conclusions can be reached.

III. OBSERVATIONS ON THE ANOPHELINE FAUNA OF VIZAGAPATAM.

During the 4 years in which the author has been employed in connection with the malaria problem at Vizagapatam, a number of observations have been made which supplement the reports of the previous workers.

(A) SPECIES OF ANOPHELINES RECORDED.

During the period under discussion 19 different species of Anophelines have been found in the Vizagapatam area, in either the adult or larval stages. Some details of these catches are given in Table I.

TABLE I.

Species.	ADULTS AND LARVAE COLLECTED DURING						Areas where collected.
	1930.	1931.		1932.		1933.	
	July to Dec.	Jan. to June.	July to Dec.	Jan. to June.	July to Dec.	Jan. to May.	
1 <i>A. culicifacies</i> *	34	24	63	52	80	72	Golf ground, Municipal wells, Malakapuram, Voota Gedda, Thummidalametta, Lova Gardens, Waltair wells and geddas, Karasa area, Yerri Gedda and Gangulu Gedda.
2 <i>A. stephensi</i> *	20	19	158	32	15	33	Malakapuram, Voota Gedda, Municipal wells, Waltair wells and geddas, Lova Gardens and Gangulu Gedda.
3 <i>A. fluviatilis</i> *	nil	nil	4	nil	6	nil	Voota Gedda, Lova Gardens, Malakapuram and hill streams near Gopalapatam.
4 <i>A. aconitus</i> *	nil	nil	3	nil	2	nil	
5 <i>A. minimus</i> *	nil	nil	3	nil	1	nil	
6 <i>A. jeyporiensis</i>	1	nil	nil	nil	nil	nil	Lova Gardens.
7 <i>A. tessellatus</i>	9	nil	3	nil	nil	nil	Thummidalametta, Malakapuram and Karasa area.
8 <i>A. varuna</i> *	nil	nil	nil	nil	nil	11	Lova Gardens.
9 <i>A. hyrcanus</i>	1	nil	nil	nil	nil	nil	Thummidalametta well.
10 <i>A. splendidus</i> *	nil	1	nil	4	nil	nil	Lova Gardens and Golf Course stream.
11 <i>A. theobaldi</i> *	nil	nil	15	nil	nil	nil	Lova Gardens, Malakapuram and Karasa.
12 <i>A. annularis</i>	2	nil	33	nil	nil	nil	Lova Gardens, Malakapuram and Karasa.
13 <i>A. jamesi</i>	2	nil	5	14	nil	nil	Lova Gardens and Gopalapatam side.
14 <i>A. karwari</i>	2	nil	nil	2	nil	6	Lova Gardens, Malakapuram and Voota Gedda.
15 <i>A. maculatus</i> var. <i>willmori</i> *	nil	1	nil	nil	nil	nil	Malakapuram.
16 <i>A. subpictus</i>	All these species are very common and occur throughout the year.						
17 <i>A. vagus</i>							
18 <i>A. barbirostris</i>							
19 <i>A. pallidus</i>							

* These species are the important carriers of malaria reported.

Senior-White also reported the finding of *A. moghulensis* in March 1932, but we have not been able to detect this species.

A total of 6,224 adult and larval mosquitoes were collected and identified, of which 490 were *A. culicifacies* adults and larvæ, and 367 were the larvæ of *A. stephensi*. In Graph 1 is shown the relationship of the incidence of these, the chief carrier species, to temperature, humidity, rainfall and malarial prevalence each month from July 1930 to May 1933.*

(B) CARRIER SPECIES OF ANOPHELINES.

As will be seen from Table I the two most prevalent carrier anophelines recorded in this locality, during the period of our observations, were *A. culicifacies* and *A. stephensi*.

A total of 1,851 adult anophelines were captured in nature among which were 48 specimens of *A. culicifacies* and one of *A. fluviatilis*. These specimens were dissected but none were found infected.

(C) SPECIAL BREEDING PLACES OF CARRIER ANOPHELINES.

(1) WELLS.

Fifty-six wells were examined in the Fort, Sivalayam, Godevari and Chengalrowpeta wards of Vizagapatam town. Out of these 30 were found to be breeding *A. stephensi*, in 2 the larvæ of *A. culicifacies* were present, and both species occurred in 4 other wells.

The wells in the Lova Gardens breed both *A. culicifacies* and *A. stephensi*, while in those on the Golf Course and at East Point only the former species was found. During 1930 and 1931, some of the wells dug by workmen at Thummidalametta (Harbour area) were found to be breeding *A. culicifacies* only.

(2) GEDDAS.

The following geddass and streams were found to be breeding potential carriers of malaria :—

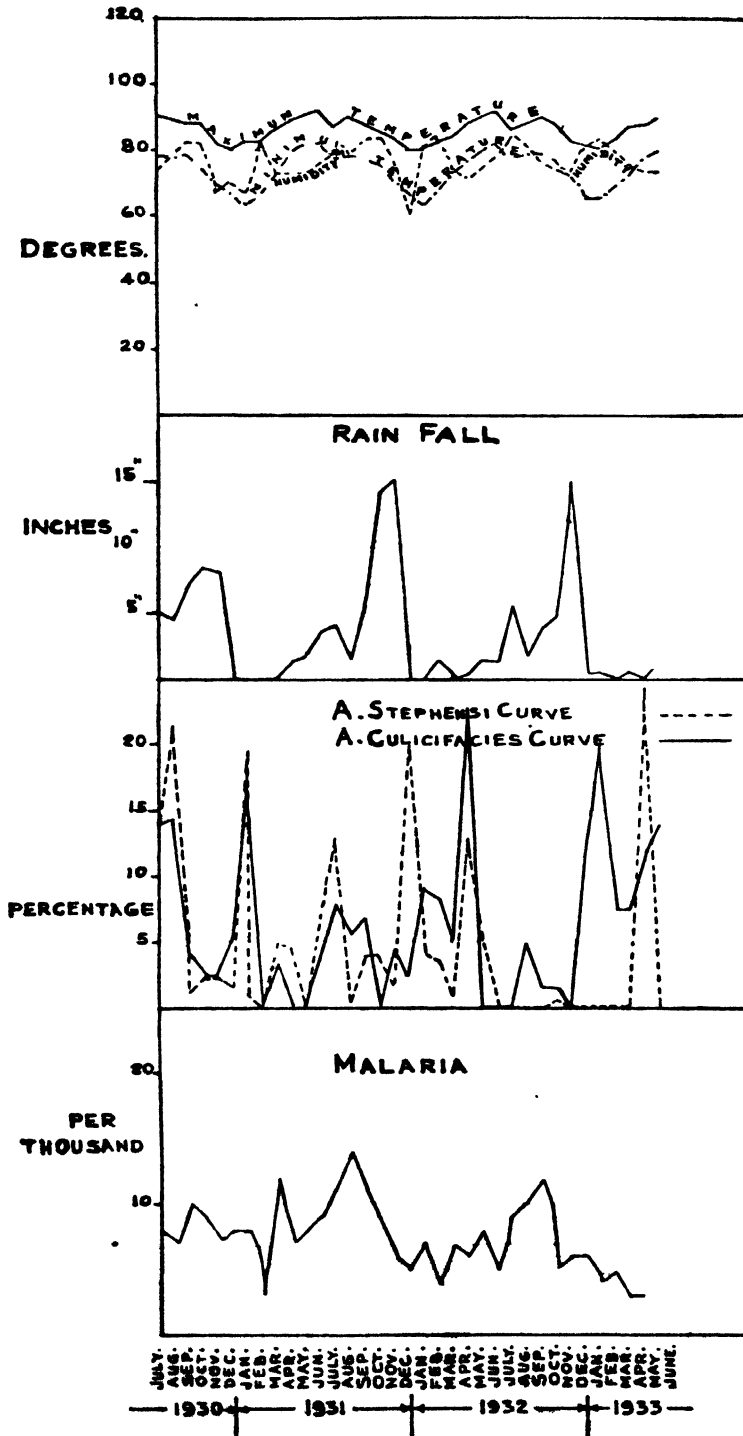
- (i) Voota Gedda—*A. culicifacies*, *A. stephensi* and *A. fluviatilis*.
- (ii) Gangulu Gedda—*A. culicifacies* and *A. stephensi*.
- (iii) Yerri Gedda—*A. culicifacies*.
- (iv) Golf Course stream—*A. stephensi*, *A. culicifacies* and *A. splendidus*.
- (v) Geddass on eastern slopes of Waltair plateau—*A. culicifacies* and *A. stephensi*.

Domestic wells and geddass were found to be a continual source of danger. From our observations, one gathered the impression that *A. culicifacies* is the most common malaria-carrying anopheline in the locality north of the Voota Gedda, while in parts of the town south of this *A. stephensi* predominates. There are, however, no actual statistics to support these impressions.

* In estimating the percentage of these species shown in Graph 1, the total number of adult and larval mosquitoes caught and identified each month were taken into account. The catches were made in controlled as well as uncontrolled areas, and in the Municipal and other areas, as well as in the Harbour area.

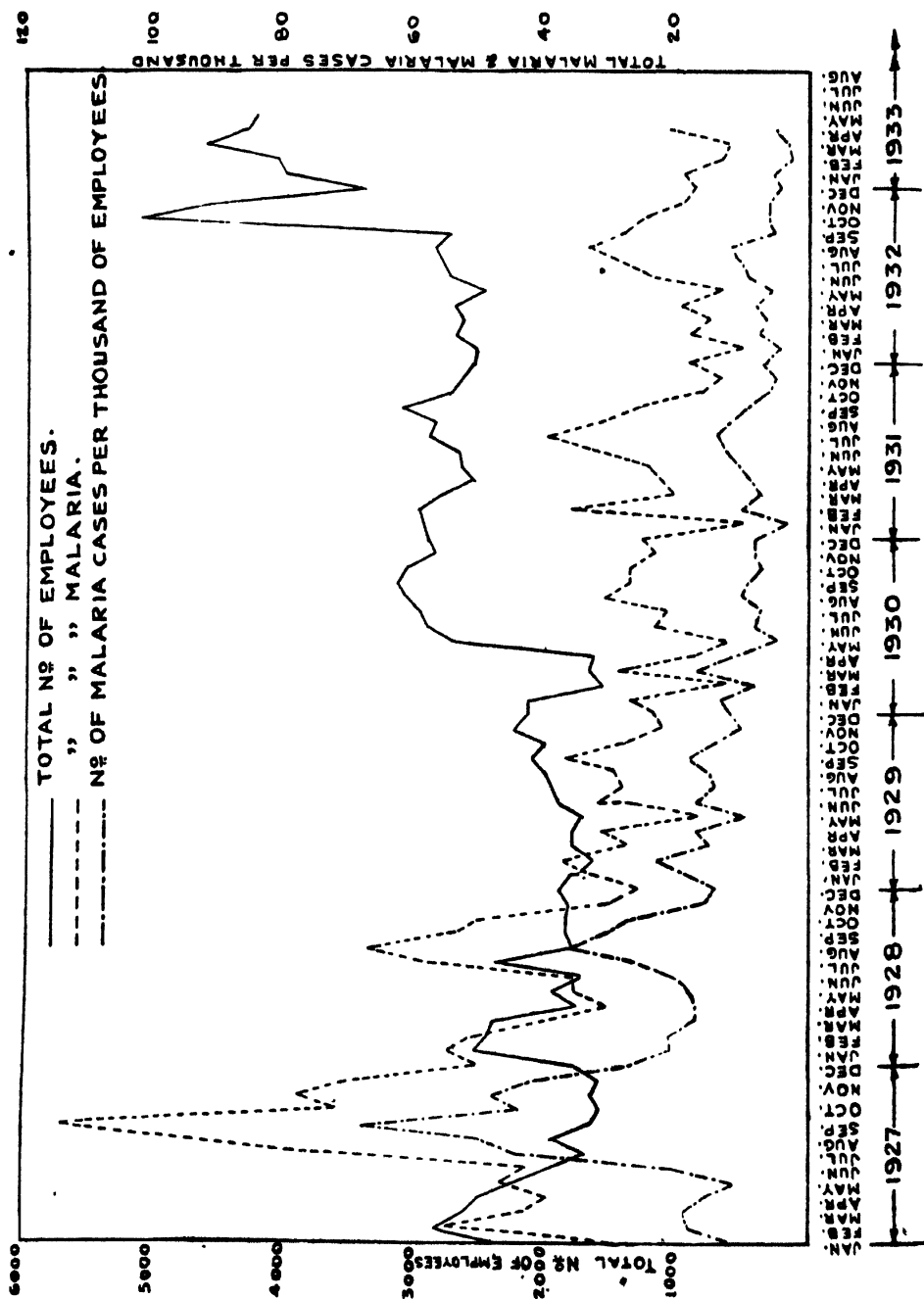
GRAPH 1.

Illustrating maximum and minimum temperatures, relative humidity, rainfall in inches, percentage of *Anopheles culicifacies* and *A. stephensi* and number of malaria cases per thousand of employees.



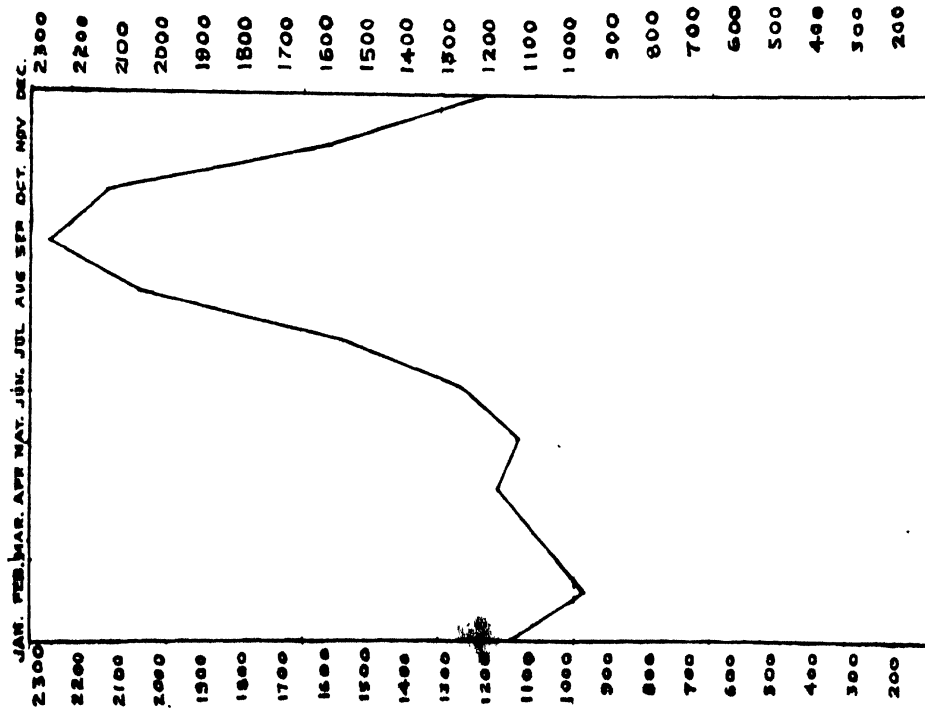
GRAPH 2.

Illustrating the total number of employees in relation to total malaria cases and malaria cases per thousand of employees.



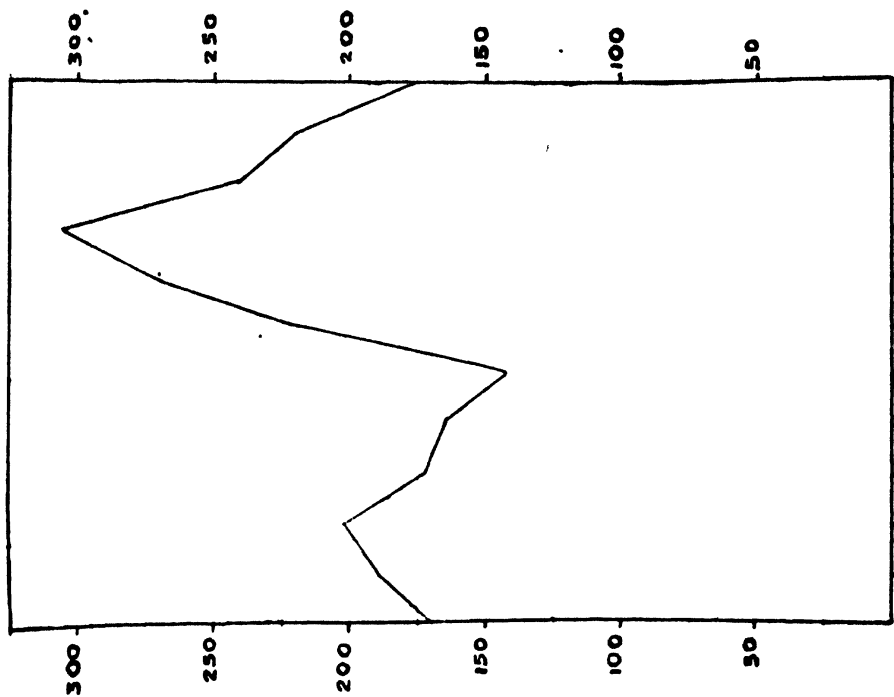
GRAPH 3.

Illustrating the seasonal incidence of malaria in Vizagapatam compiled from records of the Vizagapatam Town Dispensary. 1927-1932.



GRAPH 4.

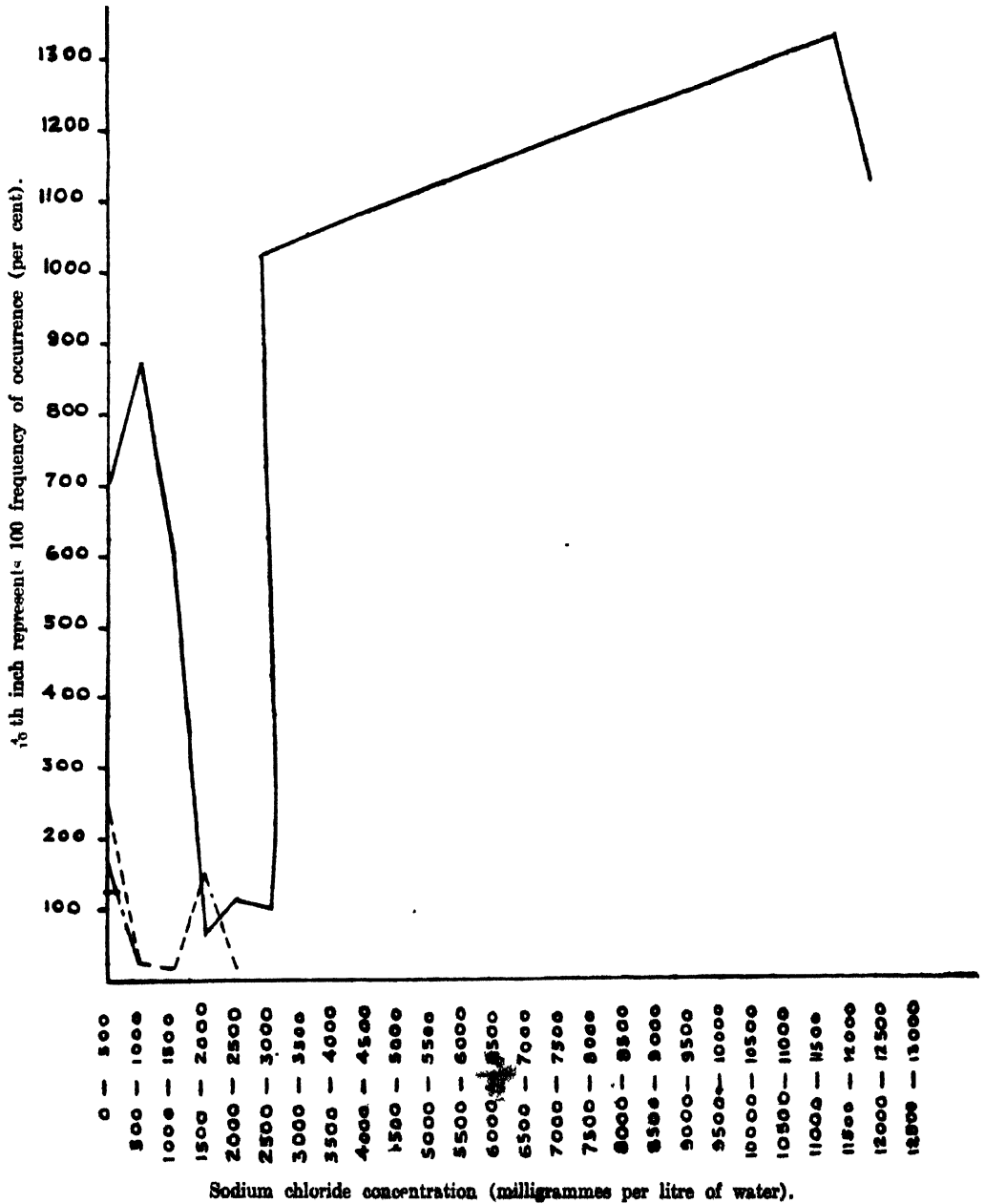
Illustrating the seasonal incidence of malaria in Vizagapatam compiled from records of the Vizagapatam Harbour Dispensary. 1927-1932.



GRAPH 5.

Illustrating the relative frequency of *A. stephensi*, *A. culicifacies* and *A. subpictus* in waters of different salt concentrations.

— *A. SUBPICTUS*.
 --- *A. STEPHENSI*.
 - - - *A. CULICIFACIES*.



(3) BRACKISH WATER.

The relative frequency with which the larvæ of various species of anophelines were collected in waters showing different degrees of salt concentration has been worked out. Graph 5 illustrates the relative frequency of the larvæ of *A. stephensi*, *A. culicifacies* and *A. subpictus* in waters of different salt concentrations.

In addition to the results recorded in Graph 5 the following species were found to breed in water showing concentrations between 0 and 500 mg. of sodium chloride per litre :—*A. pallidus*, *A. annularis*, *A. barbirostris*, *A. jamesi*, *A. splendidus*, *A. maculatus* var. *willmori*, *A. karwari* and *A. fluviatilis*.

IV. THE INCIDENCE OF MALARIA IN VIZAGAPATAM.

(A) TYPE OF INFECTION.

The relative frequency with which the different species of malarial parasite were recorded among patients at the Vizagapatam Harbour Dispensary are shown in Table II.

TABLE II.

Year.	Benign tertian per cent	Malignant tertian per cent	Quartan, per cent.	Mixed, per cent.
1927 (from 13th July, 1927).	73.0	25.0	2.0	nil
1928	76.0	23.0	1.0	nil
1929	66.3	31.1	nil	2.6
1930	56.0	41.3	1.3	1.3
1931	66.0	26.8	7.2	..
1932	78.2	16.1	4.9	0.8
1933 (up to May 1933)	75.0	22.9	nil	2.1

(B) SEASONAL PREVALENCE OF MALARIA

The seasonal incidence of malaria, as prepared from the records of the Municipal Town Dispensary of Vizagapatam from 1927 to 1932, is shown in Graph 3. Similar records from the Vizagapatam Harbour Dispensary are shown in Graph 4. From a study of these graphs it is evident that the 'malaria season' begins in the latter half of July, and from this time the incidence rises steadily to reach a maximum which usually occurs in September. Senior-White also recorded the maximum incidence as being in September in the year 1925. August, September and October are the three most malarious months.

The relationships of malarial prevalence to rainfall, relative humidity and temperature have been shown in Graph 1.

350 *Anti-Malarial Operations in Vizagapatam Harbour.*

(C) STATISTICS OF MALARIAL INCIDENCE AT THE VIZAGAPATAM HARBOUR DISPENSARY.

Details of the malarial patients treated at this dispensary are given in Table III.

TABLE III.

	1927.	1928.	1929.	1930.	1931.	1932.
Total number of new admissions	6,804	7,748	8,604	10,677	10,511	14,101
Total malaria cases ..	769	559	347	268	294	249
Percentage	11.3	7.21	4.03	2.41	2.79	1.76

During the years 1930, 1931 and 1932 the cases were grouped according to the localities from which they were drawn. These are shown in Table IV.

TABLE IV.

Analysis of malaria cases.

	1930	1931	1932
Labour residing within Municipal limits :—			
Alipur ward	38	15	8
Waltair ward	7	10	8
Maharanipeta ward ..	13	19	19
Market ward	15	9	15
Chengalrowpeta ward ..	12	12	19
Sitaraniaswamy Temple ward ..	6	11	15
Godevari ward	7	5	11
Sivalayam ward	9	15	6
Fort ward	66	75	59
Labour residing outside Municipal limits ..	67	104	79
Labour residing in the Harbour area	28	19	12
TOTAL ..	268	294	249

The statistics available have been analysed and the case incidence per 1,000 employees has been calculated. This has been shown in Graph 2.

V. SUMMARY OF ANTI-MALARIAL OPERATIONS.

The Harbour Authorities commenced in 1927 an intensive anti-malarial campaign in their own interests and in that of their neighbours in the Harbour area. A general description of the work in the different areas is given below.*

A. ANTI-LARVAL MEASURES.

(1) THE SOUTH SHORE.

This locality had been surveyed by many workers and was considered to be the most malarious area in the vicinity of the Harbour. This area comprises the valley of the Lova Gardens of about 60 acres in extent, and the Malakapuram area, of which about 312 acres were included in the anti-malarial operations. These two areas are separated by a large hill—the Yarada Hill. Large numbers of *A. culicifacies*, *A. fluviatilis*, *A. aconitus* and *A. minimus* were found breeding in both these areas, while *A. varuna* and *A. jeyporiensis* were also reported from the Lova Gardens.

(a) *Tunnel through the Yarada Hill.*—A tunnel about 1,400 feet long was constructed through this hill at a cost of about Rs. 70,000. This diverted the stream which arises in the rocky ravine at the head of the Lova Gardens and flows through them. The water supply provided by this tunnel yields about 55,000 gallons *per diem*. The diversion of this stream has undoubtedly reduced the number of breeding places and water collections in the lower parts of these gardens. This permanent work was combined with temporary measures, such as oiling, paris-greening† and drainage in the valley itself. These works have prevented any outbreak of malaria among the crews who were working day and night adjacent to the lower end of the Gardens.

(b) *Anti-malarial cut-off drain.*—This channel starts at No. 7 Gedda and, running parallel to the base of the Yarada Hill, enters into the creek at Tepparevu Bay. It is flushed daily by the tide up a distance of about half a mile from the creek. All the seepage along the lower portions of the north side of the Yarada Hill is intercepted and collected in this drain. Its construction has resulted in a considerable diminution in the level of the subsoil water in its neighbourhood. No. 7 Gedda is about one mile distant from the working centre of the anti-malarial operations. Since this drain was constructed the lateral drains proposed in some of the original schemes have been found to be unnecessary. Parts of this drain are liable to silting, and large amounts of deposit (40,000 to 50,000 cubic feet) have occasionally to be removed from it.

(c) *Evacuation of villages.*—The inhabitants of the villages of Tepparevupalem, Valikampeta, Chintalapalam and Old Malakapuram were evacuated from their homes on this shore. This removed a very malarious population which acted as a dangerous source of infection to the labour force of the Harbour works.

(d) *Miscellaneous operations.*—About 169 wells were filled up in the Malakapuram area. Much jungle and prickly pear was cleared away. The

*The reader is referred to Maps I and II for the position of the various localities mentioned.

†A short description of the organisation, methods and larvicides employed is given in Appendix II.

two geddas (Nos. 3 and 6), which showed a permanent flow of water throughout the year, were cleared of vegetation and oiled weekly. The portions of these geddas in the swampy area along the shore were trained and brought under tidal influence, and drainage channels were cut to reduce the marshiness of this locality.

(e) *Results*.—As a result of the above measures no adult anophelines could be collected in the mosquito catching stations in the Lova Gardens nor at the Dredging Khalasis' Quarters. In the water collections which remained in the area, breeding appeared to be absent, except for small larvæ one or two days old. Senior-White, during a visit in March 1932, collected larvæ of *A. moghulensis*, *A. theobaldi*, *A. varuna*, *A. culicifacies*, *A. karwari* and *A. jamesi* in the Lova Gardens. The breeding was, however, very sparse and the larvæ were all small. This worker expressed the view that the area was effectively protected by oiling measures in force.

It had been proved experimentally (Satyanarayana, 1934) that anophelines bred in the Lova Gardens crossed the channel at the mouth of the Harbour and invaded the habitations on the south side of the Darga Hill. The incidence of malaria among the population of the latter area was at one time very high.

In Table V is shown the incidence of malaria in this place as compared with that on the northern side of the hill, which is outside the limits of the Harbour control and where *A. stephensi* and *A. culicifacies* are breeding in wells. These figures show that the incidence of malaria in the uncontrolled northern area is now higher than in the southern area which has been protected by the works in the Lova Gardens.

TABLE V.

Average number of malaria cases attending the Harbour dispensary.

Year.	Amongst the Harbour employees living in the Fort ward on the northern side of the Darga Hill—an untreated area.	Amongst the people residing on the southern side of the Darga Hill—a treated area*.
1930	5.50	0.08
1931	6.25	0.03
1932	4.91	0.50
1933 (January to May) ..	3.40	0.60

Note.—The numbers of the population of Harbour employees living on both the sides of the Darga Hill are almost the same according to the census taken by me in the year 1930.

* This is the place where a village, Zalarupeta by name, once existed, the inhabitants of which were compelled to evacuate it on account of the high incidence of malaria.

(2) AREA TO THE WEST OF THE QUAY WALL.

This locality is about 594 acres in extent and is under tidal influence throughout the year, the average range of the tide being 5 feet. The areas intervening between the Quay Wall on the east and the Meghadri Gedda on the west are inaccessible to the tides. Potential breeding places in this area have been regularly watched and oiled as a protection against the anticipated 'ludlowi' (*sundaicus*) danger. Now that the site for the oil tank depôt and some of the area west of the Quay Wall have been reclaimed, there is no danger from this side.

In 1930 and 1931 there was a potentially dangerous breeding place still further west at Thummidalametta, where the contractor's coolies were quarrying. These workmen had dug wells around the foot of the hill to obtain drinking water, and these wells were breeding *A. culicifacies*. This breeding was controlled by the use of petrol. The wells have since been filled up and, except for a small amount of oiling required for lateral drains around the hill, there appears to be no serious menace from this direction.

(3) THE NORTH SHORE.

(a) *The Galli Hill*.—Some borrow-pits were created when stones for the break-waters were being quarried. These were carefully watched, but only a few larvæ of *A. subpictus* were found. The whole of this area is completely under tidal influence and no grave danger is anticipated from it.

(b) *Karasa area*.—A portion of this area about 121 acres in extent, lying adjacent to Railway land and between Municipal land on the east and District Board land on the south, is under anti-malarial control to a certain extent. This is about three-quarters of a mile from the site at present occupied by the coolie camp, which is situated beside the Railway line at the northern end of the Eastern Reclamation area. Although *A. culicifacies* has occasionally been found in this area, *A. annularis* (*A. fuliginosus*) and *A. subpictus* are the usual anophelines present. On the western boundary of the Karasa area is a gedda which flows through the middle of the village of Nawabpeta. This gedda, which lies within the jurisdiction of the District Board, breeds thousands of *A. culicifacies*. Many employees of the Harbour live in the adjacent village.

(c) *The Yerri Gedda*.—The tail end of this gedda touches the Harbour land. This part, which is under tidal influence, has never been found breeding anophelines, but in the upper parts of this stream *A. culicifacies* breed. This portion is being treated by the Railway Authorities for the protection of their colony.

(4) THE EASTERN RECLAMATION AREA.

This area lies between the shipping berths and Vizagapatam town. From the latter it is separated by the Waltair-Vizagapatam Railway line.

Malariologists anticipated that unless proper precautions were taken during the dredging and filling operations for the reclamation of this area, conditions for anopheline breeding analogous to those found during the Back Bay Reclamation Scheme at Bombay might develop (*vide* Chalam, 1924). An area of 185 acres has been reclaimed.

The dredged material was largely sand, and the control of mosquito breeding was extremely easy. Only in an area of about 10 acres did cracks develop, and breeding in these has been controlled by filling these with sand. The only anopheline found breeding in this area was *A. subpictus*, and that very rarely.

Any breeding in drains, borrow-pits, pools or scrap-iron materials in this area has been regularly watched for, and dealt with immediately. Efficient tidal communications into embanked areas were established by drainage channels.

The Gangulu Gedda coming from the Municipal area drains into the pool at the extreme end of this reclamation area. Just at its outlet from the culvert, this gedda has been found to be breeding *A. culicifacies* and *A. stephensi*. A peculiarity observed here was that the larvæ were lying in the film of water on the sand adjacent to the water edge, and were therefore very difficult to collect into a ladle. A similar phenomenon was observed at the Voota Gedda. Breeding here has been reduced to a minimum by constant tidal flushing combined with the control of fishing leases, and the use of paris green or oil.

(5) THE WALTAIR AREA.

Although this area lies within Municipal limits, the Voota Gedda is oiled weekly in the interests of the Harbour employees who live in the Harbour Park at Waltair. This gedda breeds *A. fluviatilis (listoni)* in rock fissures and *A. stephensi* in the stream. The water collections in and around East Point and the B. N. Railway Rest House are also regularly treated by oiling and paris-greening.

(6) MISCELLANEOUS.

Apart from the systematic treatment of definite areas, a constant watch had to be kept for the appearance of larvæ in new and unexpected situations. Some of these were due to the unauthorised digging of wells and borrow-pits, interference with the trap doors of tidal communications by fishermen, buffaloes undoing oiling work, washermen creating pools, etc., etc. During construction operations a watch must be kept on cement vats, stagnant pools, iron pots, silt traps, defective drains, etc.

B. MEASURES AGAINST ADULT MOSQUITOES.

A mixture composed of carbolic acid, kerosene and petrol was sprayed regularly in the Harbour offices as an insecticide.

C. COST OF ANTI-MOSQUITO MEASURES.

The average expenditure per annum for oiling and paris-green work, and towards the cost of the supervision staff, is about Rs. 5,000, as shown below :

Average amount spent annually.

1. For supervision staff	about Rs. 1,200
2. For labour	about Rs. 2,800
3. For materials	about Rs. 1,000

TOTAL Rs. 5,000

This sum is estimated to be equivalent to an average of Rs. 2-7-0 spent annually for each employee. This, however, does not include capital expenditure.

D. EFFECTS OF ANTI-MOSQUITO OPERATIONS ON MALARIAL INCIDENCE.

It will be seen from a reference to Table III and to Graph 2 that there has been a very distinct decrease in the incidence of malaria among the Harbour employees since anti-malarial measures were instituted.

The construction of harbours in several other tropical areas in the east, where conditions resembling those seen in Vizagapatam were present, has resulted in very disastrous outbreaks of malaria. Special anti-malarial precautions were taken at Vizagapatam to prevent such an occurrence, and during the period of construction no such disaster has occurred.

VI. SUMMARY AND RECOMMENDATIONS FOR FUTURE WORK.

(A) THE SOUTH SHORE OF THE HARBOUR.

Continued oiling and paris-greening are required in this area. The anti-larval measures which are carried out up to a mile from the Bay (up to No. 7 Gedda) have proved very efficient in controlling malaria there. This area includes the very malarious site evacuated by the villagers of Tepparevupalem. The conditions are now so different that the construction of residential quarters up to a line drawn through Soonametta might be permitted.

(B) THE MUNICIPAL AREA.

Anti-malarial measures are very necessary in the parts of the town immediately adjoining the Harbour area. The Fort, Godevari, Sitaramaswamy Temple, Market and Sivalayam wards, all within a radius of about half a mile from the Port, should be under efficient and organised malarial control. Wells in these places should be treated each week with petrol.

At the suggestion of Lieut.-Colonel J. R. D. Webb, I.M.S., late Director of Public Health, Madras Presidency, a scheme for an effective anti-malarial campaign in the municipality has been drawn up jointly by the Municipal Health Officer and the Harbour Medical Officer. The estimate for this scheme came to Rs. 12,747 per annum and includes the use of petrol on all wells, domestic or public.

If this sum cannot be found, at least, the minimum that should be done is (a) the filling or closing of all unused public and domestic wells, (b) the treatment of all wells in the southern part of the town with paris green weekly for four months each year (July to October), (c) the systematic oiling of geddas for the same period and (d) the quinisation of labour working with either public or private bodies.

(C) RECLAMATION OF THE LARGE MUNICIPAL SWAMP.

This area lies between the Eastern Reclamation area of the Harbour and the Market ward of the municipality. Large numbers of *A. subpictus* and culicines were found breeding here, and the reclamation of this area is necessary to ameliorate the mosquito nuisance in the town. The work is nearing completion, but the swamps north of this should also be filled up.

(D) CONTROL MEASURES AGAINST SPECIAL SPECIES OF MOSQUITOES.

(1) *A. SUNDAICUS* (*A. LUDLOWI*).

The original conditions in the neighbourhood of Vizagapatam Harbour were so favourable for the breeding of this very dangerous anopheline that special precautions were taken against it. Areas of brackish water which form potential breeding grounds for this insect are being most carefully watched, and tidal communication has been established with these to deter the breeding of this mosquito, should it be introduced.

Further measures to minimise the introduction of this mosquito are under consideration. These include the prohibition of air traffic from areas where *A. sundaicus* occurs, and that all shipping clearing from potentially dangerous ports will be required to produce a 'ludlowi-free' certificate before being allowed to enter the Harbour.

(2) *A. CULICIFACIES* and *A. STEPHENSI*.

These are the two most common malaria-carrying mosquitoes, the former in outlying areas and the latter in the denser part of the town. The general control measures mentioned above were designed mainly for the destruction of these species.

(3) *Aedes* spp.

Both *Aedes* (*Stegomyia*) *aegypti* and *Aedes* (*Stegomyia*) *vittatus* have been found in this area, and both are known to be capable of transmitting yellow fever. They have been found on several occasions breeding in tree holes and in water in boats, etc. If the virus of this disease be introduced either by shipping or air craft, the danger to the population will be great. Control measures in Ceylon should guard the port against the introduction of persons by aeroplanes from infected regions.

VII. THE FUTURE OF THE HARBOUR.

Systematic anti-malarial measures have been carried out in the Harbour area for the last 6 years. These have caused considerable expense, (a) directly on account of the cost of permanent works such as the tunnel through Yarada Hill, the cut-off channel, etc., and (b) indirectly by loss of revenue from, and cost of compensation for, the restriction of fishing and grazing rights, from the prohibition of wet cultivation, etc. As a result of these measures there have been no outbreaks of malaria during the period of construction of the Harbour.

Apart from the continuation of the present system of anti-malarial control the following recommendations are made for the future protection of the Harbour :—

(a) Almost every malaria-carrying species of mosquito known to occur in India has been found breeding in the Jammu Tank in the Lova Gardens. This tank is covered with much vegetation and receives all the seepage from the surrounding hills. It is recommended that the tank be either placed under tidal influence or be made into a pukka masonry tank, to abolish the mosquito

breeding. These recommendations could only be carried out if Government were to acquire the lower portion of the valley where the tank is situated.

(b) The northern part of the Darga Hill is a nidus of many endemic diseases, such as beri-beri, dysentery and malaria, and was the first area in which plague appeared among rats in the severe epidemic of 1918. This area is very congested with numerous wells in the majority of which *A. culicifacies* and *A. stephensi* breed. It is recommended that this area be acquired by Government and its insanitary conditions removed.

ACKNOWLEDGMENTS.

This article has been published by the kind permission of O. B. Rattenbury, Esq., Engineer-in-Chief, Vizagapatam Harbour Construction, who has kindly written a foreword.* During the period under review the Engineer-in-Chief was W. C. Ash, Esq., and to him as well as to Lieut.-Colonel F. J. Anderson, I.M.S., and Major John Ebdon, I.M.S., I wish to express my thanks.

My thanks are also due to Lieut.-Colonel G. Covell, I.M.S., and to R. Senior-White, Esq., F.R.S.E., for the help they have given me in acquiring a knowledge of various aspects of malariology.

I am deeply indebted to Dr. C. Ramamurty, B.A., M.B., B.S., B.Sc., and to Dr. V. K. Narayana Menon Ayl, M.B., B.S. I also wish to thank the staff of the Harbour Dispensary and the anti-malarial operations for the collection of statistics and for the interest they have shown in the experiments.

APPENDIX I.

FOREWORD BY O. B. RATTENBURY, ESQ., ENGINEER-IN-CHIEF, VIZAGAPATAM HARBOUR CONSTRUCTION.

Vizagapatam must always have been a malarial locality. It is surrounded by hilly country, and numerous springs and geddass occur at the base of the hills. These are favourable to the breeding of anopheline mosquitoes. Both the town and village inhabitants have constructed surface wells, generally at the foot of the hill slopes, which are also apt to harbour the larvæ of mosquitoes.

Another breeding area is along the high-water line in the swamp area inland of the town. This stretches for miles and forms a dangerous seepage band of some depth. There are also numerous tanks and borrow-pits scattered in every direction.

The intensity of malaria in the Vizagapatam District is periodic, reaching its height in September, as a result of the moderately wet weather of the south-west monsoon from June to September.

The flood rains of the north-east monsoon appear to clear out the breeding places, and the continuous dry weather experienced from November to June is unfavourable for breeding.

When the construction of the Harbour commenced in 1926, fears were expressed that there might be a great increase in the magnitude of the disease

* *Vide* Appendix I.

due to the construction operations. The Harbour Authorities consequently decided to take the necessary precautionary measures to prevent such an occurrence, and obtained expert advice from numerous sources, as described by Dr. Satyanarayana in his paper.

The objects in view were threefold. It was desirable that the city of Vizagapatam should not be adversely affected, either by the construction operations, or by the adverse conditions that might accompany the creation of the Harbour. It was necessary to take precautions to prevent a serious outbreak of malaria among the labour employed and housed by the Harbour Authorities on the site of the works. And it was desirable that the completed Harbour should be safeguarded from the breeding of anophelines, and be free of danger to shipping using the port.

Dr. Satyanarayana describes the measures taken, which are mainly of a recurring preventive nature. They have been highly successful, and there is no doubt that the construction of the Harbour with the accompanying malaria control, which has been instituted over the Harbour area, has been of great benefit to the adjoining city as far as malaria incidence is concerned.

For this great credit is due to Dr. Satyanarayana for the zeal and efficiency he has exercised in operating the preventive measures employed. He has been in control of this phase of Harbour activities ever since 1930 and his paper will, I am sure, prove of great interest and assistance to those who may be called upon to carry out control works of a similar nature elsewhere.

APPENDIX II.

ROUTINE ANTI-MOSQUITO MEASURES.

The whole of the Harbour area is divided into 8 districts, and each district is treated by anti-larval measures once weekly, and sometimes twice if necessity arises.

(A) ORGANISATION AND DUTIES OF STAFF.

(1) DUTIES OF PERSONNEL.

About 10 coolies are employed on an average in the routine operations. The Inspector, after issuing larvicides, etc., to the coolies, proceeds to the working place and allots the coolies for earthwork. He then makes a thorough search for breeding in all water collections. The search is made by taking as a standard 10 dips in each place. If any larvæ be found, they are collected into tubes and the breeding places are entered with a distinctive letter on the skeleton map of the area, which is carried. At the same time the Inspector notes the intensity of breeding in each place, by determining the number of larvæ collected in 10 dips. The breeding place is then treated by oiling or paris-greening according to its nature.

The larvæ are examined upon return to the laboratory and the species determined.

On the afternoon of the following day, the Inspector or Supervisor of the area revisits the area dealt with on the previous day. He then observes the effects of the measures carried out, and records the density of the larvæ. The

Medical Officer also pays surprise visits to the working places and the work is regularly inspected. The Chief Medical Officer also pays frequent visits of inspection and makes suggestions for the improvement of work.

(2) REPORTS.

The Inspector submits each day to the Medical Officer in charge of the work the skeleton map prepared by him, and reports upon the work performed in each area. The latter officer then issues any fresh instructions which he considers necessary.

The Inspector also submits a report each week. This is divided into columns for the record of the following data :—

- (i) Plots sprayed with larvicides and dates of spraying.
- (ii) Area treated in square yards.
- (iii) Types of water collection.
- (iv) Nature of banks, vegetation, etc.
- (v) Amount of larvicidal mixture used.
- (vi) Date and amount of mixture used.
- (vii) Density of larvæ (a) before spraying, and (b) after spraying.
- (viii) Types of mosquito breeding.
- (ix) Treatment adopted: (a) spraying, and (b) earthwork and cleaning.

Dangerous breeding places, *i.e.*, those of potential carriers of malaria, are marked in red upon the map submitted with the report. If the larvæ found be only one or two days old they are recorded as 'tiny'.

The report, after scrutiny by the Medical Officer in charge and by the Chief Medical Officer, is forwarded to the Engineer-in-Chief for his information.

(3) LARVICIDES USED IN ROUTINE WORK.

The larvicides employed in routine work were paris green, a mixture of crude oil and cresol, and petrol.

(a) *Crude oil.*

A mixture consisting of 5 gallons of crude oil, 8 ounces of castor oil and 4 lbs. of kerosene oil was tried, but was found to be expensive. A mixture of crude oil with one per cent of disinfecting fluid (phenyl) was next tested, and was also found unsatisfactory. A mixture of crude oil with one per cent cresol (non-saponified) was tried in less saline waters and a 2 per cent mixture in highly saline waters. These have now been in use since November 1930. These mixtures are sprayed from 'Four Oaks' sprayers.

Oil balls are also used in the oiling of running streams such as the Voota Gedda stream in the Lova Gardens, and similar situations. These balls are made by stitching up a mixed mass of waste cotton, Indian-corn cobs, and some sawdust in bags made of gunny cloth. The balls are about the size of a football and weigh about 4 to 5 pounds. The balls are soaked in oil for 24 hours, during which time they absorb about 2 to 3 pounds of oil. These

balls are tethered in streams, and it is noted that they supply a continuous output of oil, making a very good film on the streams and drains for about a week. They are renewed every 10 days.

(b) *Paris green.*

Experiments were conducted with different types of paris green in various proportions, using soap-stone and road dust as diluents.*

Paris green was used in one per cent, 2 per cent, 5 per cent and 10 per cent mixtures by weight in the case of soap-stone, and one per cent, 2 per cent, 3 per cent and 5 per cent with road dust. The mixing was done in a mixing machine allowing 200 revolutions to obtain a satisfactory mixture.

The soap-stone mixtures were sprayed with a Mysto Rotary Blower, No. 11, while the road-dust mixtures were distributed by hand. It was found that of the mixtures tested the best results were obtained with a 5 per cent mixture. In some highly saline waters a 10 per cent mixture was found necessary. Within 12 to 24 hours all larvæ except very tiny ones were killed. The mixture was distributed in the proportion of one pound of paris green per acre.

There appears to be some doubt as to whether the action of paris green is affected by the salinity of the water in the breeding places. In our experiments a greater strength of paris green was found necessary to obtain satisfactory results in highly saline waters than with waters of lower salinity. The difference may be due to the force of the wind, or variations in surface tension or some other unknown reason.

The treatment of wells with paris green is easy. Two or three grains of paris green per square yard mixed with soap-stone, was found to be an efficient and cheap larvicide. The fact that a continued treatment of certain wells with paris green in a 5 per cent concentration had no effect on the larvicidal fish in these wells makes one think that, in the proportions mentioned above, it should have no harmful effects on human beings if used in drinking water wells. Either soap-stone or lime may be used as a diluent under these conditions.

Experiments to determine the comparative costs of oiling and paris green were carried out. The results showed that while oiling was found to be cheaper in areas where vegetation and algæ were absent, yet paris green was cheaper in marshy areas and in places where algæ were present in large quantities.

(c) *Petrol.*

Petrol was used as a larvicide in certain drinking-water wells which were breeding *A. culicifacies*. A proportion of 4 ounces of commercial petrol (B. O. C.) per square yard is necessary to get a very good effect. This is the best method of dealing with wells, but is very expensive. It has two advantages over paris green and larvicidal fish: (a) no further check is required as to its effective action and (b) it kills larvæ in all stages and also pupæ, both of

*Details of the experiments are given in the original manuscript.

anophelines and culicines, which is not the case with paris green. As mentioned its expense is a great disadvantage.

(d) *Larvicidal fish.*

In our experience the effects produced by these have been doubtful. *Haplochilus* sp. were tried in the wells in the Lova Gardens, in the Thummidal-metta area and at East Point, but were found effective for a few days only. Mosquito breeding has been found in spite of their presence possibly because *A. stephensi* larvæ can dive to a great depth, even 20 feet. If the work of introducing the fish be left in the hands of coolies, a satisfactory result is not obtained.

(e) *Sulphate of copper.*

This was used to inhibit the growth of algæ in certain breeding places. The required quantity of copper sulphate crystals is placed in a gunny bag, and anchored at the origin of streams or drains. The bags must be shaken at intervals.

(f) *Results of observations on different larvicides.*

(i) Crude oil mixed with one per cent cresol (non-saponified) in less saline waters, and with 2 per cent in highly saline waters, was found to be effective in killing mosquito larvæ.

(ii) A 5 per cent mixture of paris green (by weight) with soap-stone as a diluent, when blown from a Mysto Rotary Blower or from the Baby Evesham Wonder Powder Sprayer, was found to be effective in killing all mature anopheline larvæ.

(iii) *A. culicifacies* was found to be breeding in rice fields, and in our limited experience paris green had no harmful effects on the rice

(iv) Petrol in the proportion of 4 oz. to the square yard of water surface was found an effective larvicide for wells.

(B) ESTIMATION OF THE EFFECTS OF MOSQUITO CONTROL.

The effects of mosquito control were gauged by the use of catching stations. For this purpose the wooden traps recommended by Strickland and Chowdhury (1930) and the crinoline traps of Richmond and Mendis described by Covell (1931) were employed. Seven catching stations were used :—

Place.	Type of trap.
1. Dredger Khalasis' Quarters ..	Crinoline.
2. Chief Medical Officer's Bungalow ..	Ditto.
3. Cooly Lines	Ditto.
4. Harbour Park	Wooden.
5. East Point	Ditto.
6. Break-waters	Ditto.
7. Lova Gardens	Ditto.

The form adopted for recording the captures in these stations had columns for the following data :—

(1) Date; (2) Captures, (a) in crinoline traps, (b) in wooden traps and (c) by hand; (3) Place where caught; (4) Species of mosquito; (5) Number dissected; (6) Initials of Collector; and (7) Remarks.

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A NOTE ON THE ANOPHELINE MOSQUITOES OF THE ANAIMALLAI HILLS.

BY

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[7th August, 1934.]

THE Anaimallais, or 'Elephant Hills', are a section of the Western Ghats, situated in the South of the Coimbatore district, and touching the adjoining states of Travancore and Cochin. The centre of the planting district which embraces nearly 40,000 acres is situated at Valparai, in latitude $10^{\circ} 20'$ North, and longitude $76^{\circ} 57'$ East.

The estates lie at an average elevation of 3,000 to 4,000 feet, and the tract is saucer-shaped—the surrounding hills rising to heights of from 5,000 to 8,000 feet, the highest point being Anaimudi 8,837 feet. The natural outlet is to the West. The tract is crossed by numerous rivers and streams, most of which are obstructed by slab rock and trees felled during the opening up of the tract. In the dry season the grassy edges of these slow-running streams, except where they are in dense shade, provide breeding places for *A. fluviatilis*. This is the only species of anopheline which we have yet found to be infected with malaria parasites. The numerous swamp breeding species appear to be harmless.

The average annual rainfall varies from 130 to 250 inches as one passes from the East to the West of the tract, the bulk of the fall occurring during the south-west monsoon. From June to October the rivers are in flood, and during this period *A. fluviatilis* larvæ cannot be found in them, though a few can be found in wells and shallow collections of water near rivers in company with *A. varuna*, a state of affairs not observed when optimum breeding

conditions obtained in the rivers. From November to February the night temperatures fall to below 60°F.

The malaria transmission season is from March until the commencement of the south-west monsoon, which usually breaks early in June. Night temperatures are then in the neighbourhood of 70°F. and conditions for mosquito breeding are ideal.

The tables given here show the results of the dissection of 1,200 specimens of anopheline mosquitoes caught in this area, and of the infection rate of *A. fluviatilis* over a period of six months.

Our thanks are due to Dr. N. Gopalan and Mr. S. N. Bhattacharjee for their valuable help in collecting specimens.

TABLE.

Record of the first twelve hundred dissections of mosquitoes, carried out in the Valparai Central Laboratory, Anaimallais, S. India.

Mosquitoes.	CAUGHT IN		TOTAL.
	Cattle sheds.	Human habitations.	
1. <i>A. annularis</i> (<i>fuliginosus</i>) ..	8	1	9
2. <i>A. barburostris</i>	8	1	9
3. <i>A. culicifacies</i>	8	8	16
4. <i>A. fluviatilis</i> (<i>listoni</i>) ..	4	199	203
5. <i>A. hyrcanus</i> var. <i>nigerrimus</i>	89	5	94
6. <i>A. jeyporiensis</i>	32	9	41
7. <i>A. jamesi</i>	33	5	38
8. <i>A. karwari</i>	374	65	439
9. <i>A. maculatus</i>	34	14	48
10. <i>A. majidi</i>	1	1
11. <i>A. pallidus</i>	20	1	21
12. <i>A. splendidus</i> (<i>maculipalpis</i>)	1	..	1
13. <i>A. subpictus</i>	55	14	69
14. <i>A. tessellatus</i>	55	2	57
15. <i>A. vagus</i>	123	12	135
16. <i>A. varuna</i>	13	6	19
TOTAL ..	857	343	1,200

All the above-mentioned mosquitoes were dissected and found negative with the exception of *A. fluviatilis (listoni)* :—

A. fluviatilis (listoni).

Months.	Number dissected.	INFECTION				Percentage infected.
		Gut only.	Gland only.	Both gut and gland.	Total.	
1934.						
January ..	7
February ..	6
March ..	6
April ..	82	5	3	2	10	12.20
May ..	73	3	2	1	6	8.22
June ..	28	2	2	7.14
TOTAL ..	203	10	5	3	18	8.86

QUANTITATIVE AND QUALITATIVE METHODS FOR DETECTION OF ATEBRIN IN URINE.

BY

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[22nd September, 1934.]

PREVIOUS WORK FOR THE DETECTION OF ATEBRIN IN THE URINE.

As a result of the introduction of atebtrin for the treatment of malaria several methods, both qualitative and quantitative, have been devised for the detection of atebtrin in urine.

For qualitative purposes, the manufacturers recommended the extraction of alkalinised urine with ether, evaporation of the ethereal extract to dryness, and resolution of the base from the residue in strong sulphuric acid. The presence of atebtrin is indicated by a yellow colour. Green (1932) recommends heating the urine, acidulated with strong sulphuric acid, and observing the characteristic yellow colour especially when looking down the tube from above. Hecht (1933) noted that a watery solution of atebtrin shows a fluorescence by ultra-violet illumination even up to a dilution of 1 in 5,000,000, and Massa (1933) has utilised the 'Woodlight' or 'Woodfilter' for demonstrating the fluorescence in solutions of atebtrin in sulphuric acid, or in 70 per cent alcohol.

These methods are only qualitative, but Tropp and Weise (1933) have elaborated a method for quantitative purposes. They extracted the alkaline urine with ether. From the extract they removed the atebtrin base in dilute sulphuric acid, and compared the resultant fluid colorimetrically with a standard solution. In control experiments in which 0.05 gm. of atebtrin was added to 500 c.c. of urine, 0.048 gm. was calculated, an error of about 4 per cent.

IMPROVED METHODS FOR THE DETECTION AND ESTIMATION OF ATEBRIN IN THE URINE.

The following methods devised by us have been found simpler and less laborious than any of the previous ones. They are also more suitable for the small clinical laboratories of hospitals.

I. TECHNIQUE OF QUALITATIVE TEST.

(1) About 100 c.c. of the urine containing atebrin are rendered alkaline with 10 gm. of potassium carbonate, and shaken with 20 c.c. of amyl alcohol in a glass cylinder.

(2) The supernatant alcohol layer is poured off from the top, and, if turbid, is washed with a saturated aqueous solution of potassium carbonate.

(3) The presence of atebrin would be evident from the typical yellow colour imparted to amyl alcohol, and can be confirmed in the following way. With a convex lens the bright sunlight is focused against a black background and the tube containing the extracted amyl alcohol interposed in a slanting position between the lens and its focus. A distinctly green fluorescence is noticeable in the beam of light, especially on moving the lens parallel to the tube. It should be distinguished from the faint blue fluorescence sometimes caused by the solution of urobilin in amyl alcohol*.

The green fluorescence, mentioned above, is quite distinctly shown in an amyl alcohol extract containing atebrin in dilutions up to 1 in 2,000,000. This last would correspond to the presence of atebrin in a dilution up to 1 in 10,000,000 in the urine tested.

II. TECHNIQUE OF QUANTITATIVE TEST.

(1) Take 100 c.c. of the urine in a separating funnel, and add 10 gm. of potassium carbonate. Shake to dissolve the latter.

(2) When this has occurred, add 20 c.c. of amyl alcohol, shake vigorously for 3 minutes, and then allow the mixture to separate into two layers.

(3) The lower urine layer is run out carefully, and the upper amyl alcohol layer is washed with 10 c.c. of a saturated aqueous solution of potassium carbonate.

(4) As a good deal of flocculent matter is present in the washed amyl alcohol, it is centrifugalised.

(5) Then 10 c.c. of the clear supernatant layer is taken in a test tube, and 2 c.c. of glacial acetic acid added.

* We have found that if one drop of pure sulphuric acid be added to every c.c. of the amyl alcohol extract, and the mixture be heated in a boiling-water bath for 3 minutes, the blue fluorescence due to urobilin is eliminated. The specimen should, however, be examined while still hot, as some turbidity appears on cooling.

The addition of quinine salts, salicylates, caffeine, plasmoquine or iron salts to the urine has not been found to interfere with the green fluorescence characteristic of the amyl alcohol extract containing atebrin.

(6) This mixture is shaken and compared colorimetrically with a standard atebirin solution.

METHOD OF PREPARATION OF STANDARD.

This is prepared by dissolving atebirin powder in amyl alcohol, which was shaken previously with a normal urine in the proportion of 20 c.c. of the alcohol to 100 c.c. of the urine made alkaline by addition of 10 gm. of potassium carbonate. The latter procedure eliminates to a great extent the error introduced by the partial solution in amyl alcohol of any pigments present in the urine. Glacial acetic acid 2 c.c. is added to 10 c.c. of the standard, as in the case of the unknown.

The atebirin powder used for preparing the standard was obtained from ampoules prepared by the makers for intramuscular or intravenous injections. Fifty mg. of the powder were dissolved in 50 c.c. of amyl alcohol and the appropriate amount added to the amyl alcohol (shaken with alkaline urine). For colorimetric purposes the standard should be so adjusted that it is not more than 50 per cent stronger than the unknown. If the standard is fixed at 10 of the colorimetric scale, the unknown should not read beyond 15 as the ratio is disturbed by fluorescence. For this purpose the standard can be diluted by amyl alcohol or a stronger standard prepared.

III. RESULTS.

It has been possible by the above method to estimate atebirin in as low a dilution as 1 in 1,000,000 in the control experiments, in which a known amount of the drug was mixed with urine (*vide* Table I). The amounts of urinary pigments soluble in amyl alcohol differ in individual urines and are liable to form a source of possible fallacy, but the error in our estimations with different specimens did not exceed 5 per cent.

TABLE I.
Control experiments (urine).

	Amount of urine.	Amount and strength of atebirin solution used.	Resulting dilution.	Dilution detected.	REMARKS.
1	100 c.c.	0.5 c.c. containing 1 mg. per c.c.	1 in 200,000	1 in 200,000	Experiment repeated 3 times.
2	100 c.c.	0.1 c.c. of 1 mg. per c.c.	1 in 1,000,000	1 in 1,000,000	Experiment repeated 6 times.
3	100 c.c.	0.1 c.c. of 0.5 mg. per c.c.	1 in 500,000	1 in 500,000	Experiment repeated 5 times.

Table II shows the results obtained from the estimation of atebirin in the urine of a monkey injected with this drug.

TABLE II.

Atebrin excreted in urine (female monkey weighing 3,650 gm.).

Day of experiment.	Amount of atebrin injected subcutaneously.	Number of mg. in urine per 100 c.c.
1st	0.05 gm.
2nd	0.05 gm.	6 mg. per 100 c.c. present in the sample of the previous 24 hours.
3rd	0.05 gm.	5.7 mg. do. do.
4th	0.05 gm.	5.7 mg. do. do.
5th	Nil.	5 mg. do. do.
6th	Nil.	4.9 mg. do. do.

This method has been tried as a means of estimating the amount of atebrin in the blood by adding 0.1 c.c. of a saturated solution of potassium carbonate to 10 c.c. of the sample and extracting with 5 c.c. of amyl alcohol. We have been able to extract atebrin from a mixture of this drug with blood, and the errors in the quantitative estimation did not exceed 10 to 15 per cent under such conditions. Further work is in progress on this subject.

SUMMARY.

A new, more simple and more delicate method has been devised for the detection and estimation of atebrin in the urine.

In the end we would like to record our thanks to Lieut.-Colonel J. A. Sinton, v.c., o.b.e., i.m.s., Director, Malaria Survey of India, under whose guidance this work was carried out, and to Dr. N. D. Kchar, d.sc., Biochemist, Malaria Survey of India, who helped us in carrying out part of the work.

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Note.—Since writing this paper the makers have introduced tablets containing 0.05 gm. of atebrin for intramuscular or intravenous injection. For preparation of the standard, one tablet should be dissolved in a few c.c. of water in a separating funnel, the solution made alkaline with saturated solution of potassium carbonate and extracted with 50 c.c. of amyl alcohol. Each c.c. of this amyl alcohol extract contains one mg. of atebrin.

ANOPHELINES INFECTED WITH MALARIA PARASITES: A FURTHER NOTE.

BY

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[10th October, 1934.]

IN a note published previously (Iyengar, 1934) the writer reported the finding of *Anopheles jeyporiensis* James (type form) infected with malaria parasites under natural conditions in Travancore State, South India. A further examination has shown that the finding relates to the varietal form of this species, namely, *A. jeyporiensis* var. *candidiensis* Koidz., and not to the type form. The writer much regrets this mistake. The type form as well as the varietal form occurs in Travancore, but the form examined from the endemic villages near Kulasekaram and found to be infected was the varietal form.

Since the reporting of the finding of three positive specimens out of 1,988 examined, a further series of dissections was carried out of *A. jeyporiensis* var. *candidiensis*. In this later series, one specimen was positive for gut infection out of 1,845 specimens examined.

Three other species of *Anopheles* were reported in the previous note (*loc. cit.*) as having been found naturally infected in Travancore, namely, *A. culicifacies* Giles, *A. varuna* Iyengar and *A. fluviatilis* James. In the subsequent series of observations on these three species, the following results were obtained :—

Species.	Number examined.	Number positive for gut infection.	Number positive for gland infection.	Total number infected.
<i>A. culicifacies</i>	708	0	0	0
<i>A. varuna</i>	62	0	0	0
<i>A. fluviatilis</i>	228	2	0	2

REFERENCE.

IYENGAR, M. O. T. (1934)

- .. Anophelines found naturally infected with malaria parasites in Travancore, *Rec. Mal. Surv., Ind.*, **4**, 1, pp. 61-63.

SOME UNSUCCESSFUL ATTEMPTS TO TRANSMIT MONKEY MALARIAL PARASITES TO COMMON LABORATORY ANIMALS.

BY

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[19th October, 1934.]

SINCE the discovery of the human malarial parasite by Laveran, many attempts have been made by different workers to transmit this infection to laboratory animals. The earlier work has been summarised by Bass (1922), and by Taliaferro and Taliaferro (1934).

The only record of a successful attempt is that of Yoshino (1926) who reported that he succeeded in transmitting *P. vivax* to young dogs, rabbits and guinea-pigs. This result does not seem to have been confirmed by any other worker.*

The recent work, which has been done on the Plasmodia of the lower monkeys, suggested that an attempt should be made to transmit infection with these parasites to other small laboratory animals. The only reference which we can find to such work is the unsuccessful attempt of Clark (1929) to transmit *P. brasilianum*, the parasite of the lower monkeys of Central and South America, to the guinea-pig.

*Many attempts to repeat the work of Yoshino (1926) have been made by Lieut.-Colonel Sinton, I.M.S., in these laboratories, but without success.

MATERIAL AND METHODS EMPLOYED.

MATERIAL.

In our work we have used three different species of *Plasmodium* isolated from natural infections in *Silenus irus* (*Macacus cynomolgus*), the crab-eating macaque of the Malay Peninsula. These *Plasmodia* are :—

(i) *Plasmodium knowlesi* Sinton and Mulligan, 1932, a parasite with a quotidian periodicity, and which produces a very rapidly fatal infection, often accompanied by hæmoglobinuria, when inoculated into the common Indian monkey, *S. rhesus*.

(ii) *Plasmodium cynomolgi* Mayer, 1907, which has a tertian periodicity, resembling *P. vivax*, and which produces only mild symptoms when inoculated into other lower monkeys; and

(iii) *Plasmodium inui* Halberstadter and Prowazek, 1907, with a quartan periodicity like *P. malariae*, and which causes very chronic infections of little severity.

TECHNIQUE.

The blood used for inoculation was withdrawn from the leg vein of an infected monkey (*S. rhesus*) into a hypodermic syringe containing a small quantity of a solution of 1·5 per cent sodium citrate in normal saline. A measured amount of this citrated blood was inoculated intraperitoneally into experimental animals.

The animals used were three rabbits, three guinea-pigs, three white rats, one white mouse, one wild rat (*Mus rattus*), three wild squirrels (*Sciurus palmarum*) and one pup. Six of these animals received a second dose of infected blood. The duration of the observation period varied from a minimum of 28 days to a maximum of 96 days. The results were in every instance negative.

In order that the infective dose of parasite should be known, the number of parasites per c.mm. of the peripheral blood of the infected monkey was first determined by the fowl-cell method devised by Sinton (1924).

During the period of observation, the blood of the experimental animals was examined with great care each day by both the thin and thick film methods.

RESULTS OF EXPERIMENTS.

EXPERIMENTS WITH *PLASMODIUM KNOWLESI*.

(A) RABBIT.

Experiment 1 (a).—This animal was inoculated with 0·75 c.c. of infected blood containing about 2,250,000 parasites.

Result.—Daily observations for 28 days by both thin and thick film methods showed no parasites.

Experiment 1 (b).—The same animal was re-inoculated five days later with 0·75 c.c. infected blood containing about 60,000,000 parasites.

Result.—Daily examinations for a further period of 63 days revealed no parasites. The total period of observation in the two experiments extended to 96 days.

(B) GUINEA-PIG.

Experiment 2 (a).—This animal was injected with 0.5 c.c. of infected blood containing about 1,500,000 parasites.

Result.—Daily examinations for 28 days revealed no parasites.

Experiment 2 (b).—The same guinea-pig was re-inoculated five days later with 0.5 c.c. of infected blood containing 40,000,000 parasites.

Result.—Daily examinations for a further period of 63 days showed no parasites in the peripheral blood. The total period of observation in the two experiments was 96 days.

(C) RATS

Experiment 3.—One white rat was given a dose of 0.25 c.c. of infected blood containing about 750,000 parasites.

Result.—Daily examinations for 28 days showed no parasites. This animal died ten days later and no parasites or malarial pigment could be detected in smears from either the liver or spleen.

Experiment 4.—Another white rat, which had previously been inoculated unsuccessfully with *P. inui* (*vide* Experiment 16), was re-inoculated with 0.25 c.c. of infected blood containing about 20,000,000 parasites.

Result.—Daily examinations were made with negative results for 23 days. At the end of this time at autopsy, smears from the liver and spleen showed no parasites nor any malarial pigment.

(D) WHITE MICE.

Experiment 5.—A mouse was inoculated with 0.16 c.c. of infected blood containing about 500,000 parasites.

Result.—Daily examinations of the blood for 28 days showed no parasites.

(E) SQUIRREL.

Experiment 6.—One squirrel was injected with 0.25 c.c. of infected blood containing about 15,000,000 parasites.

Result.—This animal died on the following day and no signs of parasites could be found in the liver or spleen.

(F) PUP.

Experiment 7.—One pup was inoculated with 2 c.c. of infected blood.

Result.—Daily examinations of the blood were made for about a month with negative results.

SUMMARY OF EXPERIMENTS WITH *P. KNOWLESI*.

One rabbit and one guinea-pig were inoculated on two occasions intraperitoneally with blood from *S. rhesus* infected with *P. knowlesi* and no infection occurred. Similar inoculations into two white rats, one white mouse, one pup and one squirrel also gave negative results.

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EXPERIMENTS WITH *PLASMODIUM CYNOMOLGI*.

(A) RABBIT.

Experiment 8 (a).—One rabbit was inoculated with 0.75 c.c. of infected blood containing 59,850,000 parasites.

Result.—Daily examinations for 16 days revealed no parasites.

Experiment 8 (b).—The same animal was re-inoculated a day later with 0.75 c.c. of infected blood containing about 2,250,000 parasites.

Result.—Daily examinations for a further period of 25 days showed no parasites in the peripheral blood. The total period of observation in the two experiments extended to 41 days.

(B) GUINEA-PIG.

Experiment 9 (a).—This animal was inoculated with 0.5 c.c. of infected blood containing about 3,990,000 parasites.

Result.—Daily examinations of blood were made for 16 days with negative results.

Experiment 9 (b).—The same animal was re-inoculated a day later with 0.5 c.c. of infected blood containing about 1,500,000 parasites.

Result.—Daily examinations for a further period of 25 days revealed no parasites by both thin and thick film methods. The total period of observation in the two experiments was 41 days.

(C) RATS.

Experiment 10 (a).—One white rat was injected with 0.25 c.c. of infected blood containing about 19,950,000 parasites.

Result.—Daily examinations of blood for 16 days showed no parasites.

Experiment 10 (b).—The same animal was re-inoculated with 0.25 c.c. of infected blood containing about 750,000 parasites.

Result.—Daily examinations of blood for a further period of 25 days showed no parasites. The total period of observation in the two experiments was 41 days.

Experiment 11.—One wild rat (*M. rattus*) was inoculated intraperitoneally with 0.25 c.c. of infected blood containing about 1,875,000 parasites.

Result.—The blood of this rat examined on the day of inoculation and the following day showed no malaria parasites but there were numerous trypanosomes present. The animal died on the third day. Smears made from the liver, spleen and lungs did not show any parasites or malarial pigment.

(D) SQUIRRELS.

Experiment 12.—One squirrel was given a dose of 0.25 c.c. of infected blood containing about 17,500,000 parasites.

Result.—The blood of this animal was examined on the next day with negative results. The squirrel died during the following night, and no signs of parasites could be found in the smears made from the liver and spleen.

Experiment 13.—Another squirrel was inoculated with 0.25 c.c. of infected blood containing about 1,000,000 parasites.

Result.—Daily examinations of blood for 15 days showed no parasites. The animal died on the 16th day. Smears made from the liver and spleen did not show any parasites or malarial pigment.

SUMMARY OF EXPERIMENTS WITH *P. CYNOMOLGI*.

One rabbit, one guinea-pig and one white rat were inoculated on two occasions intraperitoneally with blood from *S. rhesus* infected with *Plasmodium cynomolgi*, but no infection resulted. Similar inoculations into one wild rat and two squirrels also gave negative results.

EXPERIMENTS WITH *PLASMODIUM INUI*.

(A) RABBIT.

Experiment 14.—One rabbit was inoculated intraperitoneally with 0.75 c.c. of infected blood containing about 1,875,000 parasites.

Result.—Daily examinations of this animal's blood were made for 22 days but no parasites were detected. The rabbit died on the 23rd day, and smears made from the liver and spleen did not reveal any parasites or malarial pigment.

(B) GUINEA-PIG.

Experiment 15.—This animal was injected with 0.5 c.c. of infected blood containing about 1,250,000 parasites.

Result.—Daily examinations of the blood for 35 days showed no parasites.

(C) RAT.

Experiment 16.—One white rat was inoculated with 0.25 c.c. of infected blood containing about 625,000 parasites.

Result.—Daily examinations of blood were made for 35 days with negative result. This rat was re-inoculated five days later with *Plasmodium knowlesi* (*vide* Experiment 4).

SUMMARY OF EXPERIMENTS WITH *P. INUI*.

One rabbit, one guinea-pig and one white rat were inoculated intraperitoneally with blood from *S. rhesus* infected with *Plasmodium inui*, but no infection occurred.

SUMMARY.

All attempts to infect some of the common laboratory animals (rabbits, guinea-pigs, rats, mice, squirrels and a dog) by blood inoculation with the three species of Plasmodia found in the Oriental monkey, *Silenus irus*, have proved unsuccessful.

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A QUARTAN MALARIA PARASITE OF THE LOWER ORIENTAL MONKEY, *SILENUS IRUS* (*MACACUS CYNOMOLGUS*).

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INTRODUCTION.

Four different species of *Plasmodium* have been reported occurring as natural infections in the Oriental monkeys of the genus *Silenus**, namely:—

- (a) *Pl. semnopithec*i Knowles, 1919;
- (b) *Pl. inui* Halberstadter and Prowazek, 1907;
- (c) *Pl. cynomolgi* Mayer, 1907, and
- (d) *Pl. knowlesi* Sinton and Mulligan, 1932.

Of these four species the last three have been recorded as natural infections in *S. irus*.

Detailed descriptions of *Pl. cynomolgi* and *Pl. knowlesi* have been given by Sinton and Mulligan (1933a) and by Mulligan (1934). These workers have studied many pure infections with each of these two parasites, and have shown conclusively that the duration of the cycle of schizogony in the former *Plasmodium* lasts 48 hours and in the latter 24 hours.

These workers were unable to study pure infections with *Pl. inui*, so could not express any personal opinion as to its cycle. They have, however, made a careful study of the literature relating to this parasite (Sinton and Mulligan, 1933a). *Pl. inui* (sens. restr.) was described by Halberstadter and Prowazek (1907) and by Mathis and Leger (1911). None of these workers was able to determine definitely the duration of the schizogony cycle, but Mathis and Leger (1911) considered from a study of temperature charts and of blood smears that it was probably 48 hours. Leger and Bouilliez (1913) also studied a *Plasmodium* which they considered to be *Pl. inui*. They state that the cycle of this parasite was found to be 48 hours by successive blood examinations. Sinton and Mulligan (1933c) have, however, pointed out that these authors were probably dealing with mixed infections in many of their transmission experiments. Brug (1934) thinks that *Pl. inui* has probably a tertian periodicity, but it seems almost certain that this worker was studying *Pl. cynomolgi*, which, as will be shown later, is not a variety of *Pl. inui* as formerly supposed.

*Pl. semnopithec*i was described by Knowles (1919) from the blood of a langur (*Pygathrix entellus*), and later by Chimisso (1922) as *Plasmodium* sp. from *Silenus rhesus*. Neither of these workers was able to determine the duration of the schizogony cycle in the parasites studied by them.

Sinton (1934) reports the presence of a *Plasmodium* with a quartan periodicity isolated from the blood of *S. irus*. This parasite he identifies as *Pl. inui*. The present paper gives the reasons upon which this identification was based.

The only other *Plasmodium* of the lower monkeys recorded with a similar periodicity is *Pl. brasilianum* Gonder and von Berenberg-Gossler, 1908, found in monkeys from Central and South America. It is, therefore, necessary to consider whether this Oriental quartan parasite of the lower monkeys is identical

- (a) with *Pl. brasilianum*, or
- (b) with *Pl. inui* or *Pl. semnopithec*i, or

* This genus is described by different workers under various synonyms, such as *Macacus*, *Macaca* and *Pithecus*.

- (c) with some of the simian Plasmodia of other continents,* or
(d) is a new species.

ORIGIN OF THE QUARTAN PARASITE.

Sinton and Mulligan (1933b, 1933c) have pointed out the frequent occurrence of mixed plasmodial infections among the specimens of *S. irus*, which they purchased in Calcutta, and which apparently originated in the Federated Malay States. They have also discussed the fact, long recognised in mixed infections in human malaria, that at certain times one species of parasite may predominate in the peripheral blood to the complete, or almost complete, exclusion of the other.

These workers were dealing with infections in which the very virulent parasite, *Pl. knowlesi*, was present in every case. By inoculations into *S. rhesus* of very small amounts of blood from natural mixed infections in *S. irus*, they managed after many experiments to obtain an infection from which *Pl. knowlesi* was absent, and in which acute infection with *Pl. cynomolgi* appeared to be present in a pure state. A large number of rapid serial passages were made from this infection into other specimens of *S. rhesus*, and eventually they obtained an infection with *Pl. cynomolgi* of undoubted purity. This strain has been under observation for over a year and no evidence of a mixed infection has been obtained in it.

That these rapid serial passages of the original acute infection with *Pl. cynomolgi* were necessary to exclude the presence of a mixed infection, even when *Pl. knowlesi* had been eliminated, is shown by the fact that suspicion of a mixed infection with two parasites of low virulence began to be evident in some of the monkeys after about 6 months. These appearances were seen only in the animals inoculated with the first passages of the *knowlesi*-free strain, and were shown by the occurrence, in the mild chronic *cynomolgi* infection, of an occasional parasite of abnormal morphology. The number of these peculiar forms was too few upon which to make a definite pronouncement, so, when these forms were seen, the infection was sub-passaged to another *S. rhesus*.

This new animal developed what was apparently a typical acute infection with *Pl. cynomolgi*, but some months later when these parasites were very scanty and the infection chronic, the same abnormal forms were again detected. At the end of about 5 months from the original inoculation of this monkey, the infection was again sub-passaged to a fresh *S. rhesus*. In the last animal the infection obtained appeared to be a pure one of the latent parasite. The infection was rapidly passaged serially to fresh monkeys and a pure infection has been isolated. It is this parasite which we believe to be identical with *Pl. inui* Halberstadter and Prowazek, and which forms the subject of this paper.

This pure infection was isolated from *S. irus* (No. 56), the same animal from which our K₂ strain of *Pl. knowlesi* was derived. The infection has been serially passaged 6 times without any apparent change of morphology during

*The literature on all the described malarial parasites of the lower monkeys of the Old World has been reviewed in detail by Sinton and Mulligan (1932, 1933a).

a period of 9 months. The first 5 passages were through specimens of *S. rhesus* and the last through a specimen of *S. irus*. The bloods of three of these animals have been examined by differential parasite counts (*vide* Appendix) and all showed a quartan periodicity.

A careful study of the very scanty parasites, which appear periodically in a very chronic infection of an animal inoculated from the original host (*S. irus*, No. 54) of K₂ strain of *Pl. knowlesi*, has also revealed this quartan parasite. It is, therefore, evident that in one of our original hosts there was a triple infection and in another at least a double one.

These observations emphasise the difficulties, noted by Sinton and Mulligan (1933b, 1933c), in detecting mixed infections with simian Plasmodia, especially when a species is present with the characters of which one is not very familiar. It also shows the necessity of numerous rapid passages at the primary acute stage of any apparently pure infection, to exclude, as far as possible, other undetected mixed infections. As noted above, the primary passage which excluded *Pl. knowlesi* did not exclude *Pl. inui* from the acute infection with *Pl. cynomolgi*. *Pl. inui* was only excluded by the subsequent rapid sub-passages, for it was present, though undetected, in the blood of the first sub-passages.

These findings indicate that a scanty infection with one species of parasite may be almost impossible to detect in the presence of an acute infection with another one. We have only been able to isolate our quartan parasite from the mixed infection with *Pl. cynomolgi*, when the latter became very chronic and the former became the predominant, though very scanty, parasite in the peripheral blood.*

DESCRIPTION OF THE QUARTAN MALARIA PARASITE OF *SILENUS IRUS*.

The descriptions and figures have been made from a very careful study of blood preparations from specimens of *S. rhesus* infected by blood inoculation with a pure infection. These animals were used, because heavy infections develop and make it possible to study the different stages of the parasite with ease. The infections in *S. irus* produced by blood inoculation appear to be comparatively slight, but the morphology of the parasites in such animals appears identical with those seen in *S. rhesus*.

The blood films studied were stained with Giemsa's stain†, and also by the panoptic method recommended by Green (1932), *i.e.*, Leishman's stain

*Sinton and Mulligan (1933c) point out that Blanchard and Langeron (1912, 1913) were probably dealing with a mixed infection of *Pl. knowlesi* and *Pl. cynomolgi*. After our study of *Pl. inui*, we have re-examined the illustrations given by these workers, and it seems possible that they may have had a triple infection at some stage of their experiments.

†The formula of the Giemsa's stain used in azur II eosin 0·6 gm., azur II 0·16 gm., methyl alcohol 50 c.c., glycerine 50 c.c. When the stain has been thoroughly dissolved in the alcohol and glycerine, the bottle of stain is 'ripened' by placing in an incubator at 37°C. for 4 days, during which period it is occasionally shaken. After filtration the stain is ready for use. The stain is used in the proportion of 1 drop to 1 c.c. of distilled water. The latter should be alkaline to produce the best demonstration of stippling. The films are fixed in methyl alcohol, and the stain allowed to act for at least 20 minutes, preferably longer.

followed by Giemsa's stain, using distilled water buffered with phosphates as a diluent.

(1) APPEARANCES IN UNSTAINED PREPARATIONS.

The youngest forms are usually more distinct and solid-looking than similar forms of either *Pl. knowlesi* or *Pl. cynomolgi*. They are seen as rings with a large central vacuole. The amoeboidicity of these forms is sluggish, and the pseudopodia short and thick. The protoplasm of the older ring forms becomes thickened at one side of the vacuole, and it is in this thickening that the pigment is first detected as fine, yellowish brown granules. As the parasites get older, the pigment becomes more abundant and darker, with a distinct tendency to a peripheral distribution. At this stage one or two thick sluggish pseudopodia may be thrown out giving the parasite a tailed appearance. As the parasite increases in age, it becomes more rounded and appears compact and solid. The pigment is more scattered, and appears as if tending to agglomerate into larger granules of a dark brown colour. Forms with crescentic protoplasm embracing a large 'vacuole' are seen. In mature schizonts the pigment is clumped into a very dark brown or black, central mass. At no stage is amoeboidicity marked; it is always sluggish, and the pseudopodia are blunt, short and lobose.

The red cells infested by the pre-pigmented forms usually show no colour changes, but in the older forms the remains of the red cell is distinctly paler than normal.

Exflagellation has been observed, and the microgametes are about 15-18 microns long.

(2) APPEARANCES IN STAINED PREPARATIONS.

(a) ASEXUAL CYCLE.

The youngest forms appear as minute parasites about one-fourth the diameter of the infested red cell. They consist of a comparatively thick ring of deep blue protoplasm surrounding a small vacuole. The protoplasm is wider at the side of the ring opposite the chromatin mass (Plate III, figs. 1, 14 and 15), and in some instances it shows a few projections which give the parasite a stellar appearance (Plate III, fig. 15). Accolé forms are rarely seen (Plate III, fig. 14). The chromatin mass is relatively large, rounded and deeply staining. It usually projects beyond the margin of the protoplasmic ring. There is no detectable change in the size and staining reaction of the infested red cell at this stage*.

The duration of this stage is apparently short, not more than 3 hours or so, for these forms are comparatively rarely seen, and those of the next stage are very numerous. This seems to indicate that the youngest parasites quickly change their morphology.

From about the 6th to 9th hours of development, parasites about one-third the diameter of the infested cell predominate. These are commonly in the

* In chronic infections, where there is a marked anisocytosis of the red cells, one gets the impression that parasitism occurs more frequently among the microcytes than among the macrocytes. Leger and Bouilliez (1913) also noted this with *Pl. inui*.

form of large rings (Plate III, fig. 19). The protoplasm at this stage usually consists of a thin, hair-like wisp of light-blue substance surrounding a large central vacuole. Sometimes small pseudopodia are present giving the parasite a stellar or triangular appearance (Plate III, figs. 16 and 17). No pigment is visible.

The chromatin mass is large and a portion usually projects beyond the protoplasmic ring, more rarely it is seen inside the vacuole (Plate III, fig. 20). The mass stains a deep red, and is most commonly single, in which case it is rounded or quadrilateral in shape or may be crescentic or arc-like. Much less commonly the chromatin is very pleomorphic and is divided into two or three parts scattered around the periphery of the vacuole (Plate III, figs. 2—13)*. When there are two masses, these, if of equal size, tend to lie close together (Plate III, figs. 4, 7 and 8), while if unequal they are more commonly at opposite poles of the parasite (Plate III, figs. 5 and 9). Adjacent masses may sometimes be joined by a very thin thread of chromatin (Plate III, figs. 2 and 8). The relative sizes of the two masses may show all gradations from equality to two parts one of which is large and the other very small, resembling the 'accessory chromatin dot' seen in *Pl. knowlesi* and *Pl. cynomolgi* (Plate III, figs. 5 and 18). Very rarely three masses are seen (Plate III, figs. 11 and 12).

Cells infested with two parasites are not rare in heavy infections (Plate III, figs. 16, 18 and 19), while three are very uncommon. At this stage the parasitised cells are not enlarged even when they contain two or more parasites. They usually stain of a normal colour, but if two parasites be present in the same cell, there may be a trace of commencing stippling, even at this early stage (Plate III, fig. 18). Very occasionally with star-shaped forms, small pink dots may be detected at the points of the pseudopodia (Plate III, figs. 14 and 17). These possibly represent early stippling and have been noted by Brug (1934) in connection with *Pl. inuit*†.

From about the 9th to 18th hours, these rings increase in size until they become about half the diameter of the infested red cell (Plate III, figs. 20—22). At this stage the 'nuclear' vacuole is large, and sometimes traces of a 'pseudovacule' (*vide infra*) may be visible in the protoplasm. The chromatin may be rounded, elongated or arc-like. Very short, triangular or lobose pseudopodia are not uncommon and more rarely a few grains of pigment may be discerned with difficulty (Plate III, fig. 23). Some more solid looking forms are occasionally seen, which are probably young gametocytes (Plate IV, fig. 70). It is at this stage that stippling first shows itself distinctly. The background of the infested cell may at first look normal, and on it appear a few small rounded dots. The background soon becomes slightly paler than normal. The cells show no distinct enlargement.

During the next stage, lasting from about 18th to 36th hours, the parasites appear as large 'vacuolated' forms at least half the diameter of the infested red cells in size (Plate III, figs. 24—29).

*In these figures, to save space, the infested red cells have not been depicted.

†From the description given by this worker and from some preparations which he has kindly sent me, there seems little doubt that he was dealing with *Pl. cynomolgi* partly, if not entirely.

In the early part of this period large rings are common (Plate III, fig. 24). These consist of a faintly blue, protoplasmic ring surrounding a much more lightly staining central area. A few grains of pigment are present in the protoplasm peripherally. The chromatin mass is relatively large and usually marginal. Its shape varies considerably—rounded, oval, crescentic or sometimes irregular.

Before proceeding with a description of the parasites in this stage and the next, it is necessary to consider the nature of the 'vacuolation' which is such a marked feature at these periods (Plate III, figs. 24—39). In faintly stained specimens the centre of these parasites appears to consist of a very lightly staining or clear, homogeneous area, surrounded by a darker rim of blue protoplasm, and often outlined by pigment granules. In more deeply stained specimens, however, this area of 'vacuolation' is found to be made up of two portions which may, for ease of reference, be termed (a) the 'nuclear' vacuole and (b) the 'protoplasmic' or 'pseudo-vacuole'.

(a) The 'nuclear' vacuole is a rounded, colourless or very faintly stained body, always in close relationship to the chromatin mass. When the chromatin is multiple or irregular, it is usually applied to the edge of this vacuole (Plate III, figs. 26, 29, 35 and 37), and sometimes encircles it completely (Plate III, fig. 34). There seems little doubt but that this body is the achromatic portion of the vesicular nucleus of the parasite.

(b) The 'protoplasmic' or 'pseudo-vacuole' is merely a thinning of the central portion of the protoplasm of the parasite. It is in marked contrast to the deeper staining margins of the protoplasm, which are also delineated by the peripheral distribution of the pigment at this stage.

The line of demarcation between these two kinds of 'vacuoles' is usually determined with ease by critical illumination in well stained specimens, but, in poorly stained ones, the whole centre of the parasite may appear to be homogeneous.

The large rings of the early portion of this stage are usually rounded or oval with a smooth outline (Plate III, figs. 24, 26 and 29), but they soon develop small triangular or lobose pseudopodial projections (Plate III, figs. 25, 27—29). The pigment is easily discernible as fine, peripherally situated, diffuse, golden-brown granules.

As these parasites grow older, they increase in size until they are almost two-thirds the diameter of the infested red cell. They still maintain their markedly vacuolated appearance. The protoplasm often develops pseudopodial projections of a short lobose character. If single, these projections give the parasite a tailed appearance (Plate III, fig. 34), while, if double or treble, it may be T-shaped (Plate III, fig. 25) or irregular (Plate III, fig. 33). These pseudopodia are comparatively wide and blunt, and quite different from the more numerous and filamentous forms seen with *Pl. vivax* and *Pl. cynomolgi*. The chromatin is peripherally situated, and may be rounded, irregular or pleomorphic.

At this stage, in poorly stained specimens, the chromatin may sometimes appear as if disconnected from the protoplasm, because the very thin portions of the latter, surrounding the sides of the nuclear vacuole, may be very indistinct (Plate III, figs. 25, 28 and 30). Similarly, portions of the pseudopodia may not be deeply stained, so that the thickened and pigmented margins

may stand out as separate pieces, giving a false impression of filamentous pseudopodia.

Towards the end of this stage the parasites appear as large vacuolated forms with irregular nuclei and thickened protoplasmic margins, containing distinct fine pigment granules, peripherally more marked and of a brown colour.

In well stained specimens the infested red cells at the early part of this stage show a faint stippling. The general coloration of the cell may be almost normal or slightly paler. The dots are small, rounded, irregularly scattered, and purplish red. Stippling soon becomes more marked, and the general coloration of the background of the cell is distinctly paler, although the cells are usually more prominent than normal because of their stippling. The dots sometimes appear as tiny rings, or may run together in some places to form small smears (Plate III, figs. 25 and 28). They are never so distinct, large or bright red as the Schüffner's dots seen with *Pl. vivax* or *Pl. cynomolgi*. They often appear to project beyond the margin of the cell, which in the later stages may show a diffusely granulated or ground-glass-like background, as if the hæmoglobin had begun to be precipitated as very minute granules. The red cells at the end of this stage may show signs of slight enlargement.

From about the 36th to 51st hours the parasites increase in size until they are about three-quarters the diameter of the infested cell. They still show distinct 'vacuolation', and may be very pleomorphic (Plate III, figs. 30—41).

In the early part of this stage the chromatin may be a single ovoid or elongated mass, but is frequently pleomorphic (Plate III, figs. 30—34). It is sometimes divided into two parts placed on either side of the nuclear vacuole. When this occurs it may simulate precocious division (Plate III, fig. 35). The parasite, as in the previous stage, consists of a flimsy mass of protoplasm with central vacuolation. The marginal rim of protoplasm is wider, more deeply stained and more pigmented. The parasite may be oval (Plate III, fig. 38), but has often some blunt pseudopodia, giving it a tailed, triangular or irregular outline (Plate III, figs. 33—37). The pigment granules are usually fine and scattered at the edges, but some small agglomerated masses may be seen (Plate III, fig. 36).

In poorly stained specimens, as noted previously, the chromatin may appear detached from the protoplasm (Plate III, figs. 30 and 39), which may look markedly amœboid.

As the parasites increase in age their outlines become more oval, rounded or polyhedral. The protoplasm tends to stain more uniformly, so that the 'pseudo-vacuole' becomes less distinct (Plate III, fig. 41). The pigment is more abundant, is less confined to the margins of the parasite, and often tends to collect into small clumps (Plate III, figs. 39—41). The chromatin becomes more concentrated, and usually lies stretched peripherally along the edge of the 'nuclear' vacuole, which is enlarged and distinct.

The stippling of the infested cell is distinct and scattered. The cells are enlarged and may be as much as 8 microns in diameter, so that the parasite does not nearly fill them. The background of the erythrocyte is pale and the stippling may tend to obscure the margins of the parasite, thus helping to make it appear more irregular or amœboid.

From about the 51st to the 63rd hours the parasites do not increase very much in size. They have lost nearly all their pseudopodia and become oval, rounded or polyhedral (Plate III, figs. 42—44, and Plate IV, figs. 45—49). The 'pseudo-vacuole' disappears almost completely and the protoplasm stains a deep blue. The pigment is more evenly scattered in the parasite, and there is a distinct tendency for many of the fine granules to aggregate into small clumps, which show a dark or greenish-brown coloration.

The chromatin mass is large and situated peripherally on the edge of the 'nuclear' vacuole, which is larger than before. The chromatin mass may be irregular at first, but towards the end of this stage is usually compact and elongated (Plate III, figs. 42 and 44, and Plate IV, fig. 46). A very common appearance is a widely crescentic protoplasm with an elongated chromatin mass stretched between the tips of the horns of the crescent (Plate III, fig. 44, and Plate IV, fig. 46)*. In lightly stained specimens, the chromatin mass in these forms may appear as if detached from the body of the parasite, because of the almost colourless 'nuclear' vacuole which intervenes between them (Plate III, fig. 43, and Plate IV, figs. 45 and 47).

The stippling of the infested cell is distinct and in some instances the margins of these cells may be outlined by a dark reddish line, or by a row of projecting dots. The background of the cell is in some cases very pale and gives the appearance of a vacuole (Plate III, fig. 42)†.

Preparatory to true nuclear division, the most common appearance seen is for the elongated chromatin mass to send a thin projection into the 'nuclear' vacuole, so forming two crescentic bodies joined at their inner horns (Plate IV, figs. 47—49). On careful examination the 'nuclear' vacuole is seen to be divided into two parts, although no distinct separation of the chromatin into two bodies is apparent.

Segmentation proper begins about the 63rd hour and is completed about the 72nd hour of development.

As mentioned above the commencement of segmentation is indicated by the division of the nuclear vesicle into two parts, and the formation of two crescentic masses of chromatin. At this stage there appears to be little or no increase in the actual size of the parasite as seen through the microscope, and the red cell is not filled by it. The infested cell is, however, enlarged, so that the mature schizonts are as large as, or slightly larger than, the size of a normal erythrocyte.

The protoplasm is a deep blue and the pigment, which is dark in colour, is scattered through its substance, being slightly more marked peripherally. Although fine pigment granules may be seen, there is a tendency for the grains to aggregate into small masses.

The two chromatin masses separate and form a crescentic cap on each of the parts of the divided nuclear vesicle (Plate IV, figs. 50 and 51). Some of the forms seen suggest that these secondary nuclei again divide into two,

* These crescentic forms (Plate III, figs. 41 and 44) bear a very close resemblance to the 'pseudo-semilunes' described by Chmisso (1922) in *Pl. semnopitheci* as sexual forms.

† Compare with the illustrations of Mathis and Leger (1911), Plate II, figs. 29—32. This appearance appears to be very similar to that sometimes seen in the 'bib' of crescents.

forming 4 masses (Plate IV, figs. 55—59), and these again divide to form 8 nuclei (Plate IV, figs. 60 and 61). Such regular division is not usually demonstrable, and the chromatin masses may appear as irregularly scattered through a purplish or bluish, mottled protoplasm. In the mature schizonts, however, a regular peripheral arrangement of the chromatin masses is frequently seen (Plate IV, figs. 62—64), and these may assume a definite rosette or daisy-head form as in *Pl. malariae* (Plate IV, fig. 64). Occasionally in some deeply stained specimens the whole parasite may take a deep purplish colour, in which it is very difficult to differentiate the chromatin masses from the pigment (Plate IV, fig. 65).

The mature schizonts have 16 merozoites, measure about 7.5 microns in diameter, and do not completely fill the infested cell, which usually shows a narrow pale crescentic rim with distinct stippling. The stippling may project beyond the margin of the cell giving it a spinulose appearance (Plate IV, figs. 62 and 63).

The pigment during the early stages of division is seen as small dark clumps scattered throughout the parasite. It is only very shortly before maturity that it tends to collect into a central mass (Plate IV, figs. 62—64). Its colour is usually dark brown, but sometimes a greenish tinge is apparent.

The infested red cells are enlarged, but much less so than those seen with *Pl. vivax* and *Pl. cynomolgi*. The stippling, though distinct, is much less marked than with the latter parasites. Occasionally an appearance is seen in deeply stained specimens, which suggests that the stippling has become condensed to form a narrow purplish-red band around the margin of the parasite (Plate IV, figs. 57—59), or that the dots have joined together to form a filament (Plate IV, fig. 53). These appearances have been discussed by Sinton and Mulligan (1933c) and by Brug (1934). Sometimes the background of the infested cell may be almost colourless and devoid of stippling. The latter looks as if it had been condensed upon the capsule of the erythrocyte as a purplish line. This condition may give the appearance of a vacuole in the parasite (Plate IV, figs. 54, 55 and 65).

Another curious appearance, seen especially in relation to schizonts with 4 to 8 nuclei, is the occurrence of a large, oval, reddish body, which appears to be closely applied to the outside of the parasite (Plate IV, fig. 60). The exact nature of this body is uncertain. Its appearance suggests that a mass of chromatin had been ejected from the parasite during the process of schizogony, but this does not seem likely. It may possibly represent the nuclear remains of another parasite which had attacked the same cell and then degenerated, or it may be of a somewhat similar nature to the reddish-purple filaments mentioned above.

The duration of the cycle of schizogony was determined from the examination of blood slides taken every three or four hours for about 100 hours. The technique and rationale of this method have been described in Appendix. By this means the cycle was found to last for 72 hours.

(b) SEXUAL CYCLE.

Some young parasites are seen which are about one-third to one-half the diameter of the infested cell, and which may be developing gametocytes

(Plate IV, figs. 70 and 71). These forms are often elongated or sausage-shaped, and stain a deeper blue than asexual parasites of the same size. The chromatin is large, and the nuclear vacuole is usually less distinct than in asexual parasites. The pigment grains are darker in colour and more evenly distributed through the protoplasm than in asexual forms of the same age.

Macrogametocytes.—The mature forms are slightly larger than a normal red cell, and are usually intracellular. They do not fill the infested cell, which is larger than normal. Sometimes the remains of this cell are very pale or ghost-like, but show some stippling (Plate IV, figs. 74–76).

The protoplasm is a deep blue and filled with scattered, dark brown pigment granules in small clumps. Individually these appear larger than in the schizonts, more evenly distributed, and more uniform in size. Although they may be slightly more numerous towards the margins of the cell, this distribution is much less marked than in the asexual forms. The larger and more uniform size of these masses, combined with their distribution, makes them appear more numerous than in schizonts.

The chromatin is large, compact and of a deep red colour. It is rounded or elongated. In immature forms a 'nuclear' vacuole may sometimes be seen, but this is usually comparatively small (Plate IV, figs. 72–74) and tends to disappear as maturity is reached (Plate IV, figs. 75 and 76).

Microgametocytes.—These are distinctly smaller than the female forms. The protoplasm stains a light reddish purple, and in mature forms, which have been deeply stained, it is often difficult to distinguish the chromatin mass from the protoplasm. The latter is filled with small, evenly distributed pigment masses, which have a yellowish-brown tint, and are lighter in colour and slightly larger than in the macrogametocyte (Plate IV, figs. 77–79). The chromatin is a light purplish red and appears much less compact than in the female. It is large and forms from one-fourth to one-third of the parasite. It is frequently triangular in outline, and usually peripheral in position.

The parasite is about the size of a normal red cell or slightly larger. The infested red cell is often difficult to see, and the parasite may then appear as if free in the blood. On close examination one can usually discern a spinulose appearance around the margin, which seems to be the remains of the collapsed cell closely attached to the parasite (Plate IV, figs. 78 and 79).

(3) PATHOGENICITY

A. In *Silenus irus*. In natural mixed infections in this monkey no clinical symptoms were apparent, and on blood examination only *Pl. knowlesi* or *Pl. cynomolgi* could be detected with certainty. When a pure infection was inoculated into a normal specimen of this species of animal no symptoms were produced. Parasites were detected after 12 days from the time of inoculation, but they never became numerous.

B. In *Silenus rhesus*. In mixed infections of this parasite with either *Pl. knowlesi* or *Pl. cynomolgi*, one of the latter Plasmodia has always been found to predominate in the blood during the acute stages of the infection. Any symptoms observed at this time were due to the effects of these parasites. In our experience of these mixed infections, the quartan parasite has only been

recognised with certainty when the infection has lasted 4 or 5 months and become very chronic*. In pure infections with this parasite, the incubation period after blood inoculation into six specimens of *S. rhesus* varied from 5 to 15 days, with an average of about 8½. These animals showed no apparent clinical manifestations, nor did we detect any marked febrile reaction. The parasites at the height of the primary acute infection were numerous (about 90,000 per c.mm.). The numbers diminished in a week or so, and a very chronic infection resulted. In this, parasites in scanty numbers have been detectable in the peripheral blood on practically every day for 7 or 8 months.

Anisocytosis is a common, probably invariable, condition in the blood, and polychromasia accompanies it.

The infection is easily transmissible by blood inoculation to other specimens of *S. rhesus* and *S. irus*. All attempts to infect Anophelines have so far proved unsuccessful.

DISCUSSION OF THE IDENTITY OF THE PARASITE.

The following species of *Plasmodium* have been reported as occurring in the blood of monkeys and apes—

- (a) *Pl. vivax* (Grassi and Feletti, 1890);
- (b) *Pl. malariae* (Laveran, 1881);
- (c) *Pl. (Laverania) falciparum* (Welch, 1897);
- (d) *Pl. (Laverania) reichenowi* Sluiter, Swellengrebel and Ihle, 1922;
- (e) *Pl. pitheci* Halberstadter and Prowazek, 1907;
- (f) *Pl. brasilianum* Gonder and Berenberg-Gossler, 1908;
- (g) *Pl. kochi* (Laveran), 1899, with its varieties
 - (i) *Pl. kochi* var. *bouilliezi* Leger, 1922,
 - (ii) *Pl. kochi* var. *joyeuxi* Leger, 1928, and
 - (iii) *Pl. kochi* var. *macfieii* Sinton and Mulligan, 1932;
- (h) *Pl. knowlesi* Sinton and Mulligan, 1932;
- (i) *Pl. cynomolgi* Mayer, 1907;
- (j) *Pl. semnopitheci* Knowles, 1919; and
- (k) *Pl. inui* Halberstadter and Prowazek, 1907, with its variety
Pl. inui var. *gonderi* Sinton and Mulligan, 1932.

The first three species are the human malaria parasites, which some observers report as causing infections among the anthropoid apes. *Pl. reichenowi* and *Pl. pitheci* were also described from the blood of the latter animals. Although many attempts have been made by different workers to transmit these parasites to the lower monkeys, no successful results have been recorded. This fact, together with various morphological differences, makes it appear very unlikely that any of these species are identical with our quartan parasite.

*It is possible that if we had been as familiar with the morphological characters of this parasite as we are now, such mixed infections might have been detected earlier.

As the remaining six species have been found as natural infections in the lower monkeys of the New or Old World, it is necessary to consider them in greater detail.

(1) *PL. BRASILIANUM*.

Except this species, no other *Plasmodium* of the lower monkeys has been reported to have a quartan periodicity. It has only been recorded in natural infections from monkeys of Central and South America belonging to the family CEBIDÆ. Attempts to transmit the infection to *Silenus rhesus* were unsuccessful (Clark, 1930).

This parasite was originally described by Gonder and Berenberg-Gossler (1908) and by Berenberg-Gossler (1909). It was again reported upon by Clark (1930, 1931) and by Taliaferro (1932).

The recent work of Taliaferro and Taliaferro (1934) gives a detailed account of the appearances presented by this parasite in different kinds of American monkey (family CEBIDÆ).

The descriptions and illustrations given of this species of *Plasmodium* by various workers, differ from our parasite in the following particulars:—

- (a) it only produces 8–12 merozoites as a rule, rarely 16;
- (b) mature schizonts do not appear to show rosette forms;
- (c) marked vacuolation appears to be rare;
- (d) the pigment described is coarser and darker, and does not seem to have such a marked peripheral distribution in the trophozoites;
- (e) gametocytes are comparatively rare in the peripheral blood;
- (f) the infested cell is said to be enlarged in the final stages of segmentation only, and no stippling has been recorded; and
- (g) attempts to infect *S. rhesus* have been unsuccessful.

If this evidence be taken into account, one does not appear justified in considering the two parasites as being of the same species. Indeed, if further study prove them identical, our conclusion would be, as discussed later, that the name *Pl. brasilianum* Gonder and Berenberg-Gossler must be considered as a synonym of *Pl. inui* Halb. and Prow., 1907.

(2) *PL. KOCHI* AND ITS VARIETIES.

This parasite has only been recorded from the lower monkeys of Africa, chiefly from the genera *Cercopithecus* and *Papio*. The duration of the schizogony cycle is doubtful, but Martoglio *et al.* (1910) state that the fever in their animals showed a tendency to a tertian periodicity.

This species and its varieties appear to differ from our parasite in the following characters—

- (a) Although these parasites have been described by many workers, yet no segmenting forms have ever been reported from the peripheral blood;
- (b) no enlargement or stippling of the infested red cells has been noted;
- (c) its morphological characters are different; and
- (d) an attempt made by Martoglio *et al.* (1910) to transmit the infection to *Silenus* sp. was unsuccessful.

From the evidence available, it is impossible to identify our parasite with *Pl. kochi* or any of its varieties.

(3) *PL. KNOWLESI*.

Although this *Plasmodium* also occurs as a natural infection in *S. irus* from Malaya, yet it differs in the following characters—

- (a) It has a 24-hour cycle of schizogony;
- (b) it produces severe and rapidly fatal infections, when inoculated into *S. rhesus*;
- (c) the merozoites number 8 to 11;
- (d) its morphology differs; and
- (e) it causes a very characteristic deformity of the infested red cells.

(4) *PL. CYNOMOLGI*.

This species of *Plasmodium*, also recorded from *S. irus*, is still considered by many workers as identical with *P. inui*, or at least a variety of that species. Sinton (1934) and Mulligan (1934) concluded from a careful study that it should be recognised as a separate species. This opinion is confirmed later in this paper.

It differs in the following characters from the quartan parasite described here :—

- (a) It has a definite 48-hour cycle of schizogony;
- (b) morphologically it is a much more amœboid parasite, producing filamentous pseudopodia at one stage of its asexual cycle;
- (c) the mature parasites are larger; and
- (d) it causes a much greater enlargement of the infested red cell, in which the stippling is redder in colour and the dots more numerous and conspicuous.

(5) *PL. SEMNOPITHECI*.

This parasite was originally described by Knowles (1919) from an Assamese langur, *Pygathrix entellus*. Chimisso (1922) found a *Plasmodium* in a specimen of *S. rhesus*, thought to have originated in India. He did not identify his parasite with any known species, but Sinton and Mulligan (1933a) consider it to be *Pl. semnopithecii*. Wenyon (1926) records this parasite in a specimen of *Presbytes pileatus* in the London Zoo, said to have come from Assam.

This parasite has been described and figured in some detail by Knowles (1919) and by Chimisso (1922). In its marked vacuolation it bears a very close resemblance to our parasite, but the descriptions differ in the following points :—

- (a) The absence of segmenting forms from the peripheral blood;
- (b) the very small size of the chromatin mass;
- (c) the unsuccessful attempts to demonstrate stippling in the infested red cell; and
- (d) the failure to transmit the infection by blood inoculation to *S. rhesus* (Chimisso, 1922).

It is recognised that these apparent differences may be due to the incomplete nature of the observations quoted, in which case our quartan parasite may eventually prove to be identical with *Pl. semnopitheci*. If this should be so, it will be seen from the later discussion that *Pl. semnopitheci* will then have to be considered as the same as *Pl. inui*, or a variety of this species.

(6) *PL. INUI*.

Most of the Plasmodia, which have been described from the lower monkeys of Asia in past years, were classified as *Pl. inui* or as *Pl. cynomolgi*. The latter species was considered by many workers to be identical with the former, or at least a variety of it. Sinton and Mulligan (1932, 1933a), in a critical review of the Plasmodia recorded from the lower monkeys of the Old World, have analysed the descriptions of these parasites given by different workers. They concluded that the only ones which could be included under the name *Pl. inui* Halb. and Prow. (sens. restr.) were—

- (i) The original species described by Halberstadter and Prowazek (1907) from *S. irus* (*M. cynomolgus*) in Java, and from *S. nemistrinus* in Borneo and Sumatra;
- (ii) the parasites reported by Mathis and Leger (1911) from *S. rhesus* and *S. lasiotis tcheliensis* in Tonkin; and
- (iii) those found by Leger and Bouilliez (1912, 1913) in a specimen of *S. irus* in the animal houses of the Pasteur Institute, Paris.

In a later paper, Sinton and Mulligan (1933c) point out that several of the old descriptions of monkey Plasmodia have apparently been based upon mixed infections with two or more species of parasite. These workers believe that, although the original infection reported by Leger and Bouilliez (1912, 1913) may have been due to *Pl. inui*, sens. restr., yet there is considerable evidence that much of the data reported by them on the pathogenicity of this parasite was based on mixed infections. We have been unable to find in the papers of Leger and Bouilliez any internal evidence to show whether the description of their parasite was based on a single infection in one animal, or whether it was made from observations on many animals, possibly with mixed infections. For this reason, although their description of *Pl. inui* is given, more reliance has been placed on those of Halberstadter and Prowazek (1907) and of Mathis and Leger (1911) for comparative purposes.

Summaries of these descriptions of *Pl. inui* are given below. These have been taken almost verbatim from the critical review of Sinton and Mulligan (1933a).

(a) DESCRIPTION GIVEN BY HALBERSTADTER AND PROWAZEK (1907).

Halberstadter and Prowazek (1907) discovered a Plasmodium in the blood of specimens of *Silenus irus* (*M. cynomolgus*) from Java, and of *S. nemistrinus* from Sumatra and Borneo. This parasite was named by them *Pl. inui*. Unfortunately the original description of this Plasmodium is not a detailed one, and consists mainly of references to the ten coloured figures which accompany that portion of their paper which refers to *Pl. inui*. The characters

of this parasite, as obtained from the description and figures* given by these workers, may be summarised as follows :—

Asexual cycle†.—Youngest forms seen were not described [figures show (i) one small ring one-fourth to one-fifth diameter of red cell, with relatively broad protoplasm and small vacuole; (b) triangular solid parasite about one-fourth diameter of red cell. Both with single, round, excentric chromatin mass and compact protoplasm]. (Another figure shows small parasite with elongated protrusion of protoplasm; although this is evidently an early growing form, it shows abundant, fine, light, brown pigment granules, and relatively large chromatin mass); other similar forms said to have been seen. Reference made to larger extracellular, markedly vacuolated forms showing early division of chromatin. (Two such forms figured showing large vacuole, fine brown pigment granules, and 3 or 4 irregular masses of chromatin). Division of chromatin early; pigment in dividing forms aggregated into clumps (figures show dividing forms smaller than red cell; immature schizonts with fine brown pigment most abundant at periphery of parasite). Mature schizonts with 12 to 16 merozoites (figure shows brown pigment collected as single, ovoid mass in centre of 12 merozoites, which are rounded, oval or pear-shaped bodies with single, round chromatin masses occupying about one-third or more of the parasite).

No additional forms seen in smears from internal organs.

Parasitised red cells apparently unaltered at all stages; no changes suggested either in description or figures; stippling never observed, although demonstrated in *Pl. pitheci* described at the same time

Sexual cycle.—Macro-gametocytes free; nucleus peripheral with distinct inner dark and lighter outer zone (figure shows roughly rounded body with protoplasm darker and bluer than in micro-gametocyte; pigment granules abundant, fine, brownish yellow scattered through protoplasm, coarser than in micro-gametocyte and more marked peripherally; chromatin excentric and showing two zones). Micro-gametocytes have protoplasm staining lightly; nucleus large and peripheral, and rich in chromatin; pigment abundant, fine, yellowish and scattered (figure shows roughly quadrilateral, extracellular body with large, deeply staining nucleus, faint protoplasm, and abundant, fine, evenly distributed yellow pigment).

Pl. inui said to differ from *Pl. pitheci* because of fainter staining of protoplasm, and appearance of abundant, fine, yellowish pigment granules; stippling never observed as in *Pl. pitheci*, and younger forms more solid.

Duration of schizogony cycle.—Not determined†.

Pathogenicity.—No apparent disturbance in health of animals infected in nature. Transmission of infection to other monkeys of the genus *Silenus* (? same species) successful, but not to orang-outangs

(b) DESCRIPTION GIVEN BY MATHIS AND LEGER (1911).

Mathis and Leger (1911), working in Tonkin, examined the bloods of forty monkeys (*S. rhesus* and *S. lasiotis tcheliensis*), and five of these were found infected with a Plasmodium resembling those of human malaria. A full description of the morphology of the parasite is given, but from which species of natural host is not indicated. These authors classify their parasite as *Pl. inui* Halberstadter and Prowazek, 1907, with which they believe

* In these descriptions of *Pl. inui*, the notes in parenthesis were made from the figures illustrating the different articles, while the rest of the description is that given by the original authors.

† Blood films stained with Giemsa's stain.

‡ The periodicity of this parasite has frequently been quoted as 48 hours from the description given by Halberstadter and Prowazek. The original, however, only states that schizogony, which was commencing when quinine was injected, proceeded uninfluenced for 48 hours.

Pl. cynomolgi Mayer, 1907, to be identical. According to the description and figures given, this parasite appears to have the following characters:—

Appearances in fresh preparations.—Parasites seen as small, round, oval or amœboid bodies. Youngest forms not pigmented. More advanced forms show pigment in fairly large and very motile granules; pigment in still larger forms only slightly motile. Exflagellation observed.

Appearances in stained preparations.*

Asexual cycle.—Youngest forms round or oval rings about 3 microns diameter, composed of a thin wisp of clear blue, unpigmented protoplasm surrounding a relatively large vesicular nucleus, which includes a voluminous vacuole; chromatin large excentric, ruby-red dot, often more than 1 micron in diameter; always excentric and situated at one pole of the parasite; sometimes find two karyosomes of equal or unequal size, these may be at same pole or at diametrically opposite ones. (Figures do not show any extreme disparity in size of two chromatin dots). Rings resemble, very much, the young forms of *P. falciparum*; double infestation of single red cell rare (figures show no alteration in size or staining of infested cell at this stage).

As development advances, parasites become piriform, triangular, oval, or hemispherical; exceptionally show definite amœboid appearance, recalling band forms of *P. malariae*. Protoplasm clear blue, filled with greenish pigment, in very fine dust-like granules giving the parasite a greenish tint. Chromatin like an elongated ring, composed of fine granules; one side may be thickened and the other thinned, so as to disappear, the karyosome is then arc-shaped; the chromatin is separated from the protoplasm by a clear space of variable size.

Segmentation of the schizont begins well before the parasite fills red cell; forms 5 microns across may have two or more chromatin masses; division of nucleus by successive mitotic divisions, not simultaneous segmentation; schizonts with 2, 4 and up to 16 nuclei encountered. Each nucleus consists of a grouping of chromatin filaments packed together, assuming an irregular star form and surrounded by a clear area; they are distributed without order through the parasite. In segmenting forms the protoplasm does not stain uniformly, it shows clear areas of greater or lesser extent; pigment in fine greenish-yellow grains, at first fairly evenly scattered, but later tending more and more to aggregate into a mass.

Mature schizonts show 16 nuclei, which have lost their prolongations, and become compact and spherical; they are arranged in a fairly regular manner at the periphery of the parasite, often resembling a daisy-head as in *Pl. malariae*. The protoplasm is condensed about each nucleus. Pigment collected in a tight central mass. Sixteen merozoites are set free, each composed of a large lilac-red nucleus surrounded by a thin wisp of clear blue protoplasm. Infested red cells said not to be enlarged at any stage of the cycle (figures show slight enlargement with some mature forms).

Sexual cycle.—In all the early stages it is impossible to differentiate gametocytes from schizonts. Adult macro-gametocytes free; typically round or slightly oval; larger than mature schizonts, and measuring 7 to 8 microns in diameter, i.e., slightly larger than a normal red blood cell. Protoplasm, dense and homogeneous, forms most of the parasite; it takes a deeper blue stain than that of the schizonts; certain forms may show a central unstained vacuole. Pigment greenish-brown colour; granules irregular, darker and a little larger than those in schizonts; fairly uniformly distributed, but slightly more abundant at periphery. Nucleus small and generally excentric, has irregular karyosome composed of a compact mass of chromatin granules; most often separated from protoplasm by an unstained or faint pink space. Young macro-gametocytes have same characters but are intracellular; the karyosome is, however, less compact and always rounded and excentric; the large colourless vacuole is joined to it and diminishes in size as the parasite grows. The protoplasm forms a demi-lune around the vacuole, and unites by its two arms at the edge of the karyosome.

* Blood films stained with Leishman's or with Giemsa's stain.

The extracellular micro-gametocytes are irregularly rounded; smaller than macro-gametocytes (about 6 microns in diameter). Protoplasm ashy blue; pigment in scattered granules of yellow colour; lighter, less abundant and coarser than in macro-gametocytes and having tendency to aggregate in streaks or clumps. Nucleus large, less dense than in macro-gametocyte, and may occupy one-third of parasite; a rounded pink mass with some darker granules.

The infested red cell shows no change in size at any stage, but stippling of the type of Schüffner's dots sometimes seen; their demonstration seems to depend upon the stain used; only seen after Leishman's and not after Giemsa's stain. (Figures show stippling of a less intense and abundant nature than seen in *P. vivax* or *P. cynomolgi*; depicted in red cells with parasites one-half to two-thirds diameter).

Schizogony cycle.—Duration not definitely determined; fever curves irregular; percentage of segmenting to other forms suggesting 48-hour periodicity.

Pathogenicity.—No symptoms in natural infections, which can only be determined by blood examinations. Infection often prolonged and shows tendency to spontaneous cure. Transmission to other monkeys of genus *Silenus* easy (? same species); some febrile reaction observed in inoculation infections, but symptoms not severe.

Mathis and Leger (1911) identified this parasite as *Pl. inui* Halb. and Prow., 1907. The only difference noted by them was the occasional occurrence of stippling, resembling Schüffner's dots, in some of the infested cells. This was never seen when Giemsa's stain was employed, which was the stain used originally by Halberstadter and Prowazek (1907).

(c) DESCRIPTION GIVEN BY LEGER AND BOUILLIEZ (1913).

Leger and Bouilliez (1913) have given a detailed description of a Plasmodium, previously found by them (Leger and Bouilliez, 1912) in the heart blood of a specimen of *S. irus* among the experimental animals at the Pasteur Institute, Paris. The original monkey, which was found to be infected, was one of a batch of five of the same species, all of which died shortly after their arrival at the Institute. The strain was, however, maintained by sub-inoculation to other monkeys, and formed the subject of two other papers (Leger and Bouilliez, 1912; Bouilliez, 1913) before the detailed description of the morphology of the parasite was published. Unfortunately this account is unaccompanied by any illustrations but, from the very full description given, the characters may be summarised as follows*:

Appearances in fresh preparations.—Parasites appear in red cells as small, round, oval or amoeboid bodies; youngest forms not pigmented and difficult to see; older forms recognised by motility of pigment; gametocytes distinguished by larger, more numerous, and very actively motile pigment granules; exflagellation not demonstrated; gametocytes and merozoites were only types of free forms seen.

Appearances in stained preparations†.

Asexual cycle.—Youngest forms seen as very small rings (1·75 microns), composed of thin wisp, unpigmented sky-blue protoplasm surrounding relatively well-developed vesicular nucleus, which is very distinct and usually rounded with ruby-red karyosome. Latter sometimes peripheral like catch on purse, sometimes in middle of colourless pseudo-vacuole; chromatin may be double, sometimes at opposite poles of parasite, sometimes close together in which case often united by thin chromatin thread. Double or triple infection of same red cell not rare, especially in heavy infections.

* As mentioned previously, some doubt exists as to whether this description was based upon a pure infection with *Pl. inui* or not (*vide* Sinton and Mulligan, 1933c).

† Leishman's stain was mostly used, but good preparations were also obtained with Laveran's and Giemsa's stains, and with iron-haematoxylin.

Growing forms rapidly become amoeboid and have most varied aspect; more rarely stay compact and band-like. Protoplasm clear blue; pigment absent or scanty. Vesicular nucleus generally very conspicuous; chromatin always abundant, position and shape very variable, often arc-shaped or rod-like.

Older growing forms become more and more rounded up; pigment granules increase in number if not in size, are fine and dust-like, giving parasite a greenish tint.

Segmentation of the chromatin begins before the parasite fills the infested cell; 12 to 16 nuclei produced, which scattered indiscriminately at periphery of parasite. Each nucleus surrounded by protoplasmic zone. Pigment at first irregularly scattered, later concentrated in small mass in middle. Finally a kind of rosette is produced with 16 merozoites, which in some cases does not fill the red cell.

The infested red cells are not enlarged or deformed by the parasite at any stage; two may be present in one cell of normal size; cells stain more deeply than normal. Stippling resembling Schüffner's dots occasionally seen after either Leishman's or Giemsa's stain. This has apparently no connection with the age of the parasite. Blood shows marked anisocytosis in advanced stages of infection; macrocytes seldom parasitised, but microcytes frequently.

Sexual cycle.—Macro-gametocytes often quadrilateral, and generally slightly larger than micro-gametocytes; protoplasm a deep blue; pigment granules finer than in male, and scattered irregularly. Nucleus rounded, stains deep red, and seems to have no vesicular areola. Micro-gametocytes rounded or polyhedral; protoplasm greyish blue, which seems due to pigment granules grouped in irregularly distributed masses. Nucleus rounded and staining red; shows more deeply staining rods in pink background, and occupying about one-fourth of parasite.

Young gametocytes easily differentiated from schizonts of same size by presence of very distinct pigment granules. Gametocytes nearly always endoglobular and often not completely filling red cell, which is of normal size. Stippling of infested red cells seen in few instances with both Leishman's and Giemsa's stains.

Schizogony cycle.—Forty-eight hours; determined by successive blood examinations.

Pathogenicity.—Very varied pathogenic effects were ascribed to this parasite, but, as pointed out by Sinton and Mulligan (1933c), many of these effects were very probably due to the presence of undetected mixed infections at different stages of the long series of experiments carried out by Leger and Bouilliez (1913).

(d) SUMMARY OF DIAGNOSTIC CHARACTERS.

From the descriptions given by these authors the most characteristic features of *Pl. inui* would appear to be—

(I) Asexual parasites.

(A) *Chromatin*.—(i) Relatively large, prominent and usually excentric at all stages; (ii) large nuclear vesicle in trophozoites; (iii) in young forms the chromatin may be divided into two equal or unequal masses; (iv) in older forms the pleomorphic arrangement of the chromatin around the nuclear vesicle may simulate precocious segmentation; (v) chromatin often arc-like and may appear as if separated from protoplasm.

(B) *Morphology*.—(i) Young rings about one-fifth to one-fourth diameter of infested red cell; (ii) marked vacuolated appearance prominent at all stages of trophozoites; (iii) amoeboidity of lobose and not of filamentous nature as seen with *Pl. vivax* or *Pl. cynomolgi*; (iv) older trophozoites more rounded; (v) mature forms do not fill infested red cell; (vi) mature schizonts with 16 merozoites, which sometimes form a rosette.

(C) *Pigment*.—(i) This appears early, in very fine, golden or light brown granules, most marked peripherally; (ii) in older forms very abundant, darker in colour and often tending to form very small clumps, still most marked at

periphery; (iii) aggregation into central mass in mature schizonts occurs very late.

(D) *Infested red cell*.—(i) Both Mathis and Leger (1911) and Leger and Bouilliez (1913) state that this is not enlarged, but some of the figures given by the former authors suggest that a slight enlargement may occur with the older forms; (ii) stippling has been found occasionally by the authors mentioned above, and the illustrations given by Mathis and Leger (1911) suggest that the dots are scantier and less prominent than is the case with Schüffner's stippling.

(II) Sexual parasites.

(A) *Macro-gametocytes*.—(i) Small compact chromatin mass with deep blue protoplasm; (ii) pigment more scattered and coarser than in trophozoites; (iii) mature forms larger than normal red cell.

(B) *Micro-gametocytes*.—(i) Large loose chromatin mass, forming about one-fourth parasite; (ii) abundant scattered pigment, lighter brown than in female.

(III) Duration of cycle of schizogony.

Uncertain, but suggested as 48 hours.

(IV) Pathogenicity.

(i) Occurs as natural infection in monkeys of the genus *Silenus* in the Dutch East Indies, Borneo and Indo-China; (ii) easily transmissible by blood inoculation to monkeys of same genus; (iii) infections cause little or no disturbance of health.

(7) CONCLUSIONS AS TO IDENTITY OF THE PARASITE.

All the characters mentioned above, except those in relation to the infested red cells and the periodicity which are discussed later, fit in very closely with the quartan parasite which we have isolated from a natural infection in *S. irus*.

In a careful study of the blood of our infected monkeys, we have been able to find practically all the forms illustrated by Halberstadter and Prowazek (1907) and by Mathis and Leger (1911). Below is tabulated a comparison between some of the figures given by these authors and the figures in the Plates of this paper.

Comparison of illustrations of *Plasmodium inui*.

Mathis and Leger (1911).	Halberstadter and Prowazek (1907).	Plates III and IV of this paper.
Figs. 4 and 23		Fig. 29.
Fig. 17		" 42.
" 25		" 70.
" 26		" 34.
" 28		" 41.
" 29		" 45.
" 33		" 61.
" 35		" 63.
Figs. 37 and 18		" 75.
Fig. 38		" 77.
" 39		" 78.
	Fig. 14	" 1.
	17	" 56.
	19	" 61.

There seemed to us no doubt that our parasite was identical with *Pl. inui* Halb. and Prow., 1907, but, to obtain further confirmation, we asked Dr. Henry Morin of Hanoi (Tonkin) if he could obtain for us specimens of the parasite described by Mathis and Leger (1911) from the monkeys of that country. Dr. Morin has very kindly sent us numerous blood slides from different animals, having both natural and inoculated infections with the monkey malaria parasites of Indo-China. We have compared these with our specimens and have been unable to detect any differences. Several of Dr. Morin's specimens, as noted by him, show distinct stippling of the type seen with our quartan parasite*. The cells infested by mature parasites also seemed larger than the average in most instances (*vide infra*).

The evidence which has been accumulated appears to leave no doubt as to the identity of our parasite with *Plasmodium inui* Halberstadter and Prowazek, 1907.

Apart from the morphological differences, the presence of a quartan periodicity shows that *Pl. cynomolgi* Mayer, 1907, can no longer be considered as a variety of *Pl. inui*, as formerly supposed. Whether *Pl. inui* var. *gonderi* Sinton and Mulligan, 1932, must still be considered as a variety of *Pl. inui* or as one of *Pl. cynomolgi*, can only be determined with certainty when this variety has been more fully studied. Its morphological characters, however, suggest a nearer relationship to *Pl. inui*.

The chief diagnostic characters of *Pl. inui* Halberstadter and Prowazek, 1907, have been described above. In Table I are contrasted the differential features of this Plasmodium with *Pl. knowlesi* Sinton and Mulligan, 1932, and *Pl. cynomolgi* Mayer, 1907, the two other malarial parasites of Oriental monkeys of the genus *Silenus*.

TABLE I.

	<i>Pl. inui.</i>	<i>Pl. cynomolgi.</i>	<i>Pl. knowlesi.</i>
Natural hosts	<i>Silenus irus.</i> <i>S. nemestrinus.</i> <i>S. rhesus.</i> <i>S. lasiotis tcheliensis.</i>	<i>S. irus.</i>	<i>S. irus.</i>
Regions from which recorded.	Borneo, Java, Sumatra, Tonkin, Malaya.	Malaya.	Malaya.
Duration of schizogony cycle.	72 hours.	48 hours.	24 hours.

* In view of these findings, the suggestion made by Sinton and Mulligan (1933c) that Mathis and Leger (1911) may have been dealing with a mixed infection appears less probable.

TABLE I—concl'd.

	<i>Pl. inui.</i>	<i>Pl. cynomolgi.</i>	<i>Pl. knowlesi.</i>
Chromatin in young ring forms.	Frequently double and of very unequal size.	'Accessory dot' present.	'Accessory dot' present.
Trophozoites.	Amœboidicity of lobose nature; vacuolation marked up to early segmentation.	Amœboidicity marked, of 'vivax' character; vacuole at first well developed but not marked in old forms.	Amœboidicity slight or absent; vacuole small in older forms.
Pigment in trophozoites.	Yellow to brown, becoming darker with age; appears early; fine and abundant with peripheral distribution.	Golden-brown; appears later and is coarser and scantier than in <i>Pl. inui</i> ; distribution less markedly peripheral.	Golden-brown to almost black; appears early; abundant.
Mature schizonts.	Maximum 16 merozoites. Often rosette.	Maximum 16 merozoites. More irregular.	Maximum 11 merozoites. Grape-like cluster.
Gametocytes.	About size of normal red cell; pigment scattered, yellowish brown to brown and abundant.	Distinctly larger than red cell; pigment not very abundant; darker than in <i>Pl. inui</i> .	About size normal red cell; pigment relatively coarse, brown to black, and abundant.
Infested red cells.	Slightly enlarged with older forms. Stippling less conspicuous, scantier than with <i>Pl. cynomolgi</i> .	Much enlarged with old forms. Stippling very conspicuous and dots very numerous.	Not enlarged; showing characteristic distortion. Stippling only shown by special stains.
Pathogenicity.	Few or no symptoms. Easily inoculable to other species of <i>Silenus</i> . Not inoculable into higher monkeys.	Usually no severe symptoms. Easily inoculable to other specimens of <i>Silenus</i> .	Mild in <i>S. irus</i> , but causing very severe symptoms, often hæmoglobinuria, when inoculated into <i>S. rhesus</i> . Has been transmitted to man and the gibbon.

The result of this work shows that *S. irus* may be infected in nature with three different species of *Plasmodium*, namely—

- (a) *Plasmodium knowlesi* Sinton and Mulligan, 1932, with a 24-hour cycle of schizogony,
- (b) *Plasmodium cynomolgi* Mayer, 1907, with a 48-hour cycle, and
- (c) *Plasmodium inui* Halberstadter and Prowazek, 1907, with a 72-hour cycle.

These parasites may occur as mixed infections, and probably very commonly do so in various combinations (Sinton and Mulligan, 1933c).

DISCUSSION OF SOME SPECIAL FEATURES IN RELATION TO THE DIAGNOSIS AND MORPHOLOGY OF *PLASMODIUM INUI*.

(1) CHANGES IN THE INFESTED RED CELL.

(a) ENLARGEMENT.

Sinton and Mulligan (1933a) have noted that the diameter of the red cells in apparently healthy specimens of *S. rhesus*, as determined in fresh and stained preparations, varied from 6.75 to 7.75 microns, the average being about 7.25 microns. It is, however, not uncommon to find a considerable degree of anisocytosis in such animals.

Leger and Bouilliez (1913) report that, with such conditions of anisocytosis, the young forms of *Pl. inui* appear to be associated more commonly with the microcytes than with the macrocytes. This has also been our experience with the quartan parasite described here, in infection with which this blood condition is very common. We have also noted it in the preparations sent by Dr. Morin from Indo-China.

Both Mathis and Leger (1911) and Leger and Bouilliez (1913) were unable to detect any definite enlargement of the infested red cells in the infections studied by them. We have also found it difficult to determine with certainty such enlargement in infections showing a marked degree of anisocytosis. We have, however, convinced ourselves that this parasite causes a distinct enlargement of the red cell which it infests. It is, therefore, necessary to explain these differences of opinion.

The enlargement begins to be evident about the time when stippling commences, and is shown in the camera-lucida drawings of Plates III and IV. This enlargement is easily recognised in heavy infections, especially where two or more broods of parasites are present. If, under such conditions, one compares the sizes of cells infested with young forms with those showing maturer parasites, the difference is evident.

In old chronic infections, where the parasites are scanty and anisocytosis is present, the change is much less strikingly shown. Here the enlarged infested cells (about 8 microns in diameter) do not appear any larger than most of the macrocytes, and may even appear smaller than some of them. This makes it very difficult, in such infections, to be certain that enlargement has taken place, unless a large number of cells infested with parasites of different ages are measured or drawn.

From our examination of chronic infections, and from a study of the comparatively scanty infections seen in the slides sent by Dr. Morin, it appears very probable that conditions of anisocytosis account for the failure of previous workers to recognise the slight cellular change. Indeed, some of the figures given by Mathis and Leger (1911) show the red cells infested with more mature parasites to be slightly larger than those with young forms (*vide* their figures Nos. 14, 15, 17, 19, 20, 25, 30-32, 35 and 37). They also note that macro-gametocytes may measure as much as 8 microns.

This evidence, we consider, suggests very strongly that the infections studied by former workers probably showed a slight, but undetected, enlargement of the infested red cells. The enlargement, however, is not so marked as that seen with infections of either *Pl. vivax* or *Pl. cynomolgi*.

(b) STIPPLING.

Halberstadter and Prowazek (1907) failed to demonstrate stippling, but this phenomenon was occasionally found by both Mathis and Leger (1911) and Leger and Bouilliez (1913). The type of stippling illustrated by the former authors closely resembles that seen in our preparations and that observed in the smears sent by Dr. Morin from Indo-China. This consists of dots which seem smaller and less well-defined than those seen with *Pl. vivax* and *Pl. cynomolgi*. They are less conspicuous, being less brightly stained and less numerous. Even with deep and prolonged staining, these dots never obscure the morphology of the parasite in the marked manner in which this may occur under similar circumstances with *Pl. cynomolgi*. Their distribution and number much more closely resemble the Ziemann's stippling seen with *Pl. malariae*.

The dots usually appear solid, but sometimes ring forms are seen. They may show distinct inequality in size and in depth of staining in the same cell. Occasionally they appear to have joined together to form short rods or threads, or to have clumped into little smears or smudges. With the older parasites, they often appear to project beyond the margin of the cell, while in some cases they seem to have condensed into a dark line, leaving a portion of the cell very pale and ghost-like*.

The filamentous bodies and dark red edges, mentioned by Sinton and Mulligan (1933c), and by Brug (1934), in connection with the mature forms of *Pl. knowlesi* and *Pl. cynomolgi*, sometimes occur. These appearances are probably connected with the phenomenon of stippling.

The difficulty, experienced by earlier workers in demonstrating stippling, was probably due to the same difficulties encountered in showing Ziemann's stippling in the human quartan parasite, *Pl. malariae*. Dr. Morin informs me that he can easily show stippling with the infections present in Indo-China, where Mathis and Leger worked. We have found it easy to place the stippling in evidence in our infections with *Pl. inui*, when the panoptic method of Green (1932) is used, or by the routine method of Giemsa staining employed in these laboratories†. It is essential, however, to use alkaline distilled water, and that in our laboratories may at times show a pH as high as 7.6.

(c) COLORATION OF THE INFESTED CELL.

During the early stages the infested cell appears to stain normally, apart from any slight suspicion of polychromasia which may be present in microcytes. This normal appearance continues usually for a short period after stippling begins to become evident. When stippling becomes more marked, the background tends to take a paler staining, and sometimes has a ground-glass appearance which gives one the impression that the hæmoglobin has begun to precipitate out as extremely minute granules.

As mentioned previously, in mature parasites one occasionally sees a clear area of red cell like a vacuole, which is bordered by a very deeply stained line of stippling.

* Vide footnote † on page 387.

† Vide footnote † on page 382.

Although the general background of the cells may be paler than normal, the occurrence of stippling often makes them more conspicuous than the uninfested red cells.

(2) DURATION OF THE ASEQUAL CYCLE.

The statement made by Halberstadter and Prowazek (1907) that schizogony, which was commencing when quinine was injected, proceeded uninfluenced for 48 hours, would be equally in keeping with a 72-hour cycle as with a 48-hour one.

Mathis and Leger (1911) thought from a comparison of the percentage of segmenting forms to other forms that the cycle was a 48-hour one. In the earlier part of our parasite counts (*vide* Appendix), the re-appearance of segmenting forms after 48 hours made us think that the observation of the latter authors was correct, but when we followed the infection by 3-hourly observations for about 100 hours, it was evident that we had been deceived in our first impression by a double quartan infection. Such a condition appears to be very common with *Pl. inui* infections, and it seems quite possible that this may have misled Mathis and Leger (1911).

(3) VACUOLATION OF THE PARASITES.

As discussed above, the characteristic 'vacuolation' seen in *Pl. inui* seems to be caused by three different conditions—

- (a) the vesicular nucleus,
- (b) the thinning of the central portions of the protoplasm in the younger growing forms of parasite, and
- (c) the ghost-like appearance of a portion of the infested red cell.

(4) SEGMENTATION OF THE PARASITES.

As the process of segmentation in *Pl. inui* takes at least 12 hours to reach completion, this species of *Plasmodium* appears specially suitable for the study of the steps in the process. This is particularly the case with the blood of *S. rhesus*, in which animal heavy infections can be produced and so a very large number of forms in various stages of segmentation can be studied.

As mentioned previously, some of the forms seen suggest that a regular process of division of each mass of chromatin into two occurs, and that this process is preceded by division of the nuclear vesicle. The question requires more detailed study.

(5) A HALO AROUND THE CHROMATIN OF THE PARASITE.

Brug (1934) mentions specially that the nuclei or chromatin bodies of young forms of *Pl. knowlesi* 'are usually surrounded by a clear, wholly unstained halo, so that there is no visible connection between protoplasm and chromatin. The halo not only separates nucleus from protoplasm of the parasite, but also extends into the red corpuscle, as far as this borders the nucleus'. This appearance he suggests 'looks as if the nucleus emanates a

substance that prevents the staining of the adjacent protoplasm of parasite and corpuscle. However, it is possible, too, that the halo is a part of the nucleus, not stained by any of the components of the Giemsa stain. Usually the halo persists as long as the nucleus remains a solid, dark red stained mass. In older parasites, where the nucleus is present as a pale red stained mass with dark red inclusions, the halo is mostly absent. After segmentation of the nucleus halos often reappear'.

The appearances described by Brug have been seen by us with *Pl. inui*, and we have also observed them with *Pl. vivax* and with *Pl. falciparum*. We are not convinced, however, that the explanations given account for the halo effect.

The halos appear most marked when the chromatin masses seem most solid and compact, as in young rings. The distinctness of the effect varies with the type of illumination used, and its size does not seem to have any connection with the edges of the vesicle of the nucleus. The opinion we formed was that the halo was probably due mainly to the optical effect of the transmitted light upon a solid and rounded mass of chromatin, rather than to any true structural feature of the parasite.

(6) THE OCCURRENCE OF BANDED FORMS OF PARASITE.

Brug (1934) has made some interesting observations upon the occurrence of band forms in films of *Pl. knowlesi* and of *Pl. falciparum*. He noted that there was a very irregular distribution of such forms in malarial films, some parts showing them in large numbers, while in other portions of the same preparations they were completely absent. These band forms were found most commonly in the very thinnest parts of such films. He considers that this can be explained tentatively as follows—'in the thicker parts of the film the parasites are killed more slowly by desiccation and so there is more time for them to assume a more or less globular form as is common with agonizing protozoa. In the very thinnest parts of the film desiccation kills the parasites instantaneously leaving them no time to take the globular form'. Brug (1934) also noted that 'all the band forms in the same field were always stretched parallel to each other, be it lengthwise or crosswise or obliquely; apparently this had nothing to do with the direction in which the upper slide was pushed when the film was prepared'.

This phenomenon was discussed by Callanan (1926) in relation to banded forms of *Pl. falciparum*. He suggests that the parallel arrangement 'is highly suggestive that banded forms are produced by a rolling between the two slides of the erythrocytes containing large ring forms. This action can be compared to the effect produced by a rolling pin on a pastry board'. He also investigated the occurrence of 'tenue forms' in smears from the East African type of *Pl. falciparum*. These forms he considers to be due to 'a combination of pressure, adhesion, surface tension and possibly other physical and mechanical conditions', which come into play while the film is being made.

The parallel arrangement of band forms of *Pl. inui* has been seen in heavy infections in our work. Figures 86 to 69 of Plate IV were drawn from parasites lying close together on a very thin portion of a blood film. The

parallel arrangement of these banded forms is noticeable, and it is also seen that this morphology occurs in all stages from young parasites up to pre-segmenting forms. As noted by Brug, this type of 'band' has never been observed by us except in the *very* thinnest parts of the films, even when the rest of the preparation showed an uncrowded and even distribution of the erythrocytes.

This parallel arrangement of the parasites suggests very strongly that some physical factor was at work in producing the result, as indicated by Callanan (1926). This factor, or combination of factors, almost certainly causes the distortion at the time when the film is being spread. Although the very elastic erythrocytes may rapidly regain their normal contour, the distorted parasites appear to be fixed in their abnormal shapes in the very thin parts of the film. That the parasites very quickly regain their usual shape is shown by their appearance in other parts of thin films. Apparently, however, the time required for this result is not sufficiently long on the very thin parts of the film, to allow the resumption of the normal morphology before the parasites become dried and fixed to the slide.

It is quite possible that some species of the malaria parasite, or some stages in the development of these species, may be more easily distorted than others, or that their rate of recovery after distortion is slower. If this be so, it may help to account for the more frequent occurrence of such forms with some species of parasites than with others. If such an explanation be correct, the 'abnormal' forms would be of diagnostic value, *not* as an indication of special morphology, but rather of a special physical state peculiar to the species of parasite.

SUMMARY.

(1) A *Plasmodium* with a quartan periodicity has been isolated from a mixed infection in a specimen of *S. irus*, said to originate in Malaya.

(2) This parasite has been compared with the descriptions of the different malarial parasites recorded from the lower monkeys, and has been identified as *Plasmodium inui* Halberstadter and Prowazek, 1907.

(3) A new and more detailed description of *Pl. inui* has been prepared.

(4) Several special characters, in relation to the diagnosis and morphology of this parasite, have been discussed.

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APPENDIX.

ESTIMATION OF THE DURATION OF THE CYCLE OF SCHIZOGONY.

It is often very difficult to determine accurately the duration of the schizogony cycle of the malarial parasites observed in the blood of infected monkeys. It has long been recognised that in human malaria the temperature chart during acute primary infections may give little indication of the true periodicity of the parasite responsible for the fever. Such charts have been found much less reliable in the case of infected monkeys, because, even in apparently normal animals, the records of temperature show very considerable daily variations. Apart from this fact, acute primary infections in experimentally infected animals may be due to one or more 'broods' of parasites reaching maturity on different days. Thus a 'double' tertian or a 'triple' quartan may give rise to a quotidian fever.

Such multiple infections are not uncommon under experimental conditions, and parasites in many different stages of development may be present in the peripheral blood at the same time. Various methods have been devised to determine the true periodicity of schizogony from an examination of the blood. The methods used by other workers have been summarised by Mulligan (1934) as follows :—

'Stephens and Christophers (1908) devised a practicable method for determining the periodicity of malarial infections in man. This method involves the making of blood smears at regular (4-hourly) intervals, and estimating, by means of a micrometer scale, the size of several hundreds of parasites observed at any given time. A preponderance of parasites of approximately the same size will be found at any particular time. According to these authors it is necessary, in order to determine the cycle of a Plasmodium, to proceed as follows :—

"1. To estimate the size and percentage of parasites of each size at any particular time, *e.g.*, starting with the onset of the attack.

2. To follow each group to its period of maximum development in the circulation.

3. To estimate the time between this period and the next appearance of young forms.

4. To estimate the time between the appearance of young forms.....and a second similar outburst".

L. G. Taliaferro (1925) used a modification of this method for the determination of the schizogony cycle of *P. cathemerium*. Outline camera-lucida drawings of a number of parasites (50) were made on squared paper at regular intervals of time. The mean measurement for the parasites present at any particular time was calculated from these drawings.

Mathis and Leger (1911), working with *P. inui*, employed the simple method of estimating the percentage of segmenting forms in proportion to other forms present over a period of several consecutive days. The accuracy of this method is open to criticism (*vide infra*).

Boyd (1929), working with *P. cathemerium*, used a similar method. This worker estimated the percentage of segmenting forms to other forms, in serial blood films made at regular (2-hourly) intervals'.

None of these methods has proved entirely satisfactory in this laboratory. The disadvantages encountered were—

- (a) while the method of measurement may give good results with more compact parasites, such as *Pl. malariae* and *Pl. knowlesi*, it was found difficult and laborious in the case of very amœboid parasites, such as *Pl. vivax* and *Pl. cynomolgi**; and
- (b) the method of counting mature schizonts may give fallacious results, if two or more infections, of approximately equal intensity and maturing on different days, are present in the blood at the same time.

The method used in this laboratory to determine the duration of the cycle of schizogony of any of the monkey malarial parasites, has been primarily to study carefully the different morphological characters presented in pure infections of the species under investigation†. One thus becomes familiar with the different appearances presented by the parasites during the progress of the cycle. When this has been done, the cycle of development is divided into a number of arbitrary stages, differentiated by the size of the parasites, their morphological characters, the changes in the parasitised red blood cell, and the time of appearance and nature of the pigment. The number of different stages employed is usually 5 or 6, and these are, as far as possible, consistent with clear differential characteristics, spaced equally throughout the cycle. The latter is unfortunately not always possible with any degree of accuracy.

In using this method, thin blood films are taken at regular short intervals, usually 3-hourly, for a number of days‡. At least 200 parasites are examined in each film and classified into the different arbitrary stages selected. The percentage of parasites belonging to these different stages is recorded separately from each film, and is charted as a graph (*vide* Chart I).

As pointed out by Mulligan (1934) the advantages of this method are—

(a) Once the observer is familiar with the morphology of the parasite, and has decided upon the differential characters of the stages to be used, it is quickly and easily carried out.

(b) It shows the periodicity of many different stages in the development of the parasite, not one only as in the case of counting mature schizonts. The periodicity of any of these different stages may be used in determining the true periodicity of the parasite, and each helps to confirm the results obtained with the others.

* Even although *Pl. imai* is not very amœboid, with it this method is not very satisfactory, because there is little apparent increase in size of the parasite, as judged by the optical section seen under the microscope, during the later half of its asexual cycle. During this period the parasite seems to increase in thickness, rather than in superficial area.

† This is an elaboration of the method used by Sinton (1922) in the study of *P. tenue*.

‡ These regular observations should be continued for at least 24 hours more than the suspected duration of the cycle of schizogony, preferably longer. This is essential, because the sudden appearance of schizonts, due to a double or treble infection, may give an appearance suggesting the completion of schizogony, although this has not taken place in the brood under observation. In the present investigation, the appearance of schizonts after 48 hours in the cycle of this quartan parasite, suggested a tertian periodicity. When, however, the observations were continued up to 96 hours the true periodicity was easily discernible (*vide* Chart I).

(c) The occurrence of double or triple infections maturing on different days is easily detected, and each of these can be followed through its cycle in the graph, thus acting as confirmation of the periodicity of the other infections.

(d) The difficulty, uncertainty and labour of measuring very amœboid forms are eliminated.

(e) The results enable a much more accurate determination to be made of the duration of each stage of the parasite.

(f) During the process of enumeration, the observer becomes thoroughly familiar with the morphology of the different stages of the parasite. This is a very great advantage if a description of the parasite has to be prepared, or if mixed infections with other species of *Plasmodia* have to be studied.

Results.

After a careful study of serial slides from infections with this simian *Plasmodium* the following arbitrary stages in the cycle of schizogony were chosen for enumeration :—

Stage I (Plate III, figs. 1—22).—Small rings less than half the diameter of the infested red blood cell, which showed no change or very faint stippling only.

Stage II (Plate III, figs. 23—29).—Parasites about one-half to two-thirds the diameter of the infested red cell, which usually shows distinct signs of stippling if intensely stained. The morphology of these forms passes from large rings into oval, tailed and vermicular forms, the last being very characteristic of the later part of this stage. Pigmentation appears early in this period, and is usually seen with little difficulty towards the end.

Stage III (Plate III, figs. 30—41).—Larger forms about three-quarters the diameter of the infested cell, which is stippled. The parasites at this stage tend to become more rounded, oval or polyhedral; pseudopodia, if present, are usually thick and short. The protoplasm is flimsy, and more deeply stained at the margins of the parasite, where the pigment is more abundant. This gives the appearance of pseudo-vacuolation, which tends to disappear as the parasite passes towards the next stage. The chromatin at this time may show a tendency to become scattered or broken, an appearance which may simulate precocious division.

Stage IV (Plate III, figs. 42—44, and Plate IV, figs. 45—49).—These large forms in many instances almost fill the infested red cell. The protoplasm stains more densely and the pseudo-vacuolated appearance has disappeared. The pigment is not so markedly marginal as in stage III. The chromatin in the earlier parts of this stage may be broken or irregular, but tends to become more compact, rounded, oval or elongated later.

Stage V (Plate IV, figs. 50—60).—These forms show true division of the chromatin up to a maximum of 6 masses.

Stage VI (Plate IV, figs. 61—65).—In this stage are grouped all schizonts having more than 6 masses of chromatin.

The results obtained by the enumeration of the different stages of the parasites, according to this arbitrary classification, are shown in Chart I.

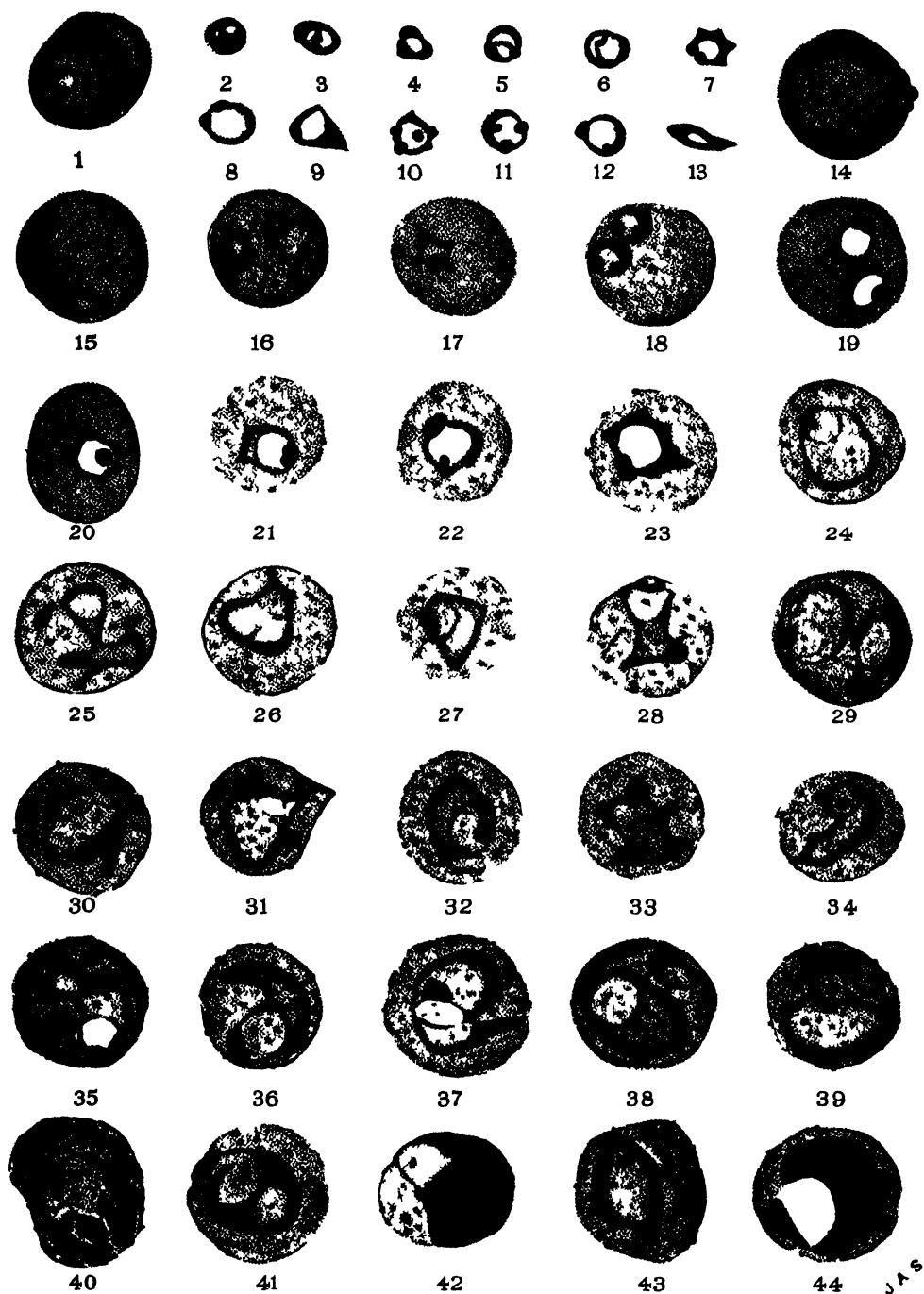
From an examination of this chart, it will be seen that, if the observations had been continued for 3 days only, the findings would strongly suggest a tertian periodicity, as reported by Mathis and Leger (1911) and by Leger and Bouilliez (1913) with *Pl. inui*. When, however, the observations were continued up to 5 days, it became evident that the results obtained were only explicable by the presence of a double quartan infection. That this was the case is supported and confirmed by the results obtained by the enumeration of all the different stages of the parasite.

Similar results were obtained in two other infections with this parasite. There is, therefore, no doubt that the Plasmodium responsible for these infections has a quartan periodicity. We believe, therefore, that *Pl. inui* Halb. and Prow. has a 72-hour cycle of schizogony and not a 48-hour one as previously reported.

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PLATE III.



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DESCRIPTION OF PLATE III.*

Illustrations made with Abbé camera-lucida, using Leitz No. 15 \times B periplanatic ocular, 2 mm. apochromatic objective and 160 mm. tube length.

Fig. 1. Very young ring form.

Figs. 2 to 13. Young parasites showing varied types of morphology of protoplasm and chromatin. (The infested cells have not been shown, so that space may be saved).

Fig. 14. A young accolé form of parasite, showing bright red dot at end of pseudopodium (*cf.* Fig. 17).

„ 15. Young parasite with stellar outline.

„ 16. Red cell with two parasites of varied shapes.

„ 17. Stellar parasite with red dots at end of pseudopodia. (These may represent early stippling).

„ 18. Double infestation of red cell; parasites with double chromatin dots. Early stippling in cell of normal colour.

„ 19. Double infestation of red cell of normal colour and no stippling.

„ 20. Stellar parasite with central chromatin mass; infested cell with normal staining properties.

Figs. 21 and 22. Larger ring forms with commencing stippling and change of colour in red cells.

Fig. 23. Large irregular ring form with few pigment granules.

Figs. 24 to 35. Larger parasites showing (i) distinct peripheral pigmentation, (ii) varied morphology, (iii) pleomorphic chromatin in close relation to 'nuclear' vacuole, (iv) 'nuclear' and 'protoplasmic' vacuolation, and (v) stippled red cells with paler background.

„ 36 to 41. Larger growing forms with (i) more marked pigmentation, (ii) varied morphology, (iii) marked 'nuclear' and diminished 'protoplasmic' vacuolation, and (iv) slight enlargement of stippled red cells.

„ 42 to 44. Large forms showing disappearance of 'protoplasmic vacuole' and enlargement of 'nuclear' vesicle. Fig. 42 shows pale area of infested cell simulating a vacuole. Figs. 43 and 44 show chromatin apparently detached from protoplasm, because of large intervening nuclear vesicle.

* In the reproduction of these plates the pigment granules have been made much too coarse, and cannot, therefore, be taken as an accurate guide to the appearances seen in stained specimens.

DESCRIPTION OF PLATE IV.*

Figs. 45 to 49. Large trophozoites prior to commencing segmentation. Figs. 45 and 47 show apparent detachment of chromatin from protoplasm. Figs. 47, 48 and 49 show division of nuclear vesicle and preparation of chromatin for segmentation.

„ 50 to 53. Show various stages in primary division of the chromatin into two parts.

„ 54 and 55. Show pale areas of infested red cell simulating vacuolation. In Fig. 55 commencing secondary division of the chromatin is indicated.

„ 56 to 59. Show continued division of chromatin.

„ 60 and 61. Show the chromatin dividing into 8 parts. In Fig. 60 the curious oval pink body of doubtful character is shown lying against the parasite.

„ 62 and 63. Show schizonts with clumping of the pigment and the remains of the infested red cells.

Fig. 64. Shows an almost mature rosette-shaped schizont without any detectable red cell.

„ 65. Illustrates a very deeply stained schizont with the remains of the red cell giving a vacuolated appearance.

Figs. 66 to 69. Show 'band' forms. These were drawn from the same part of a very thin film. Note that these forms tend to lie parallel to each other, in spite of their different stages of development.

„ 70 and 71. Young gametocytes, showing distribution of pigment and almost complete absence of 'protoplasmic' vacuole.

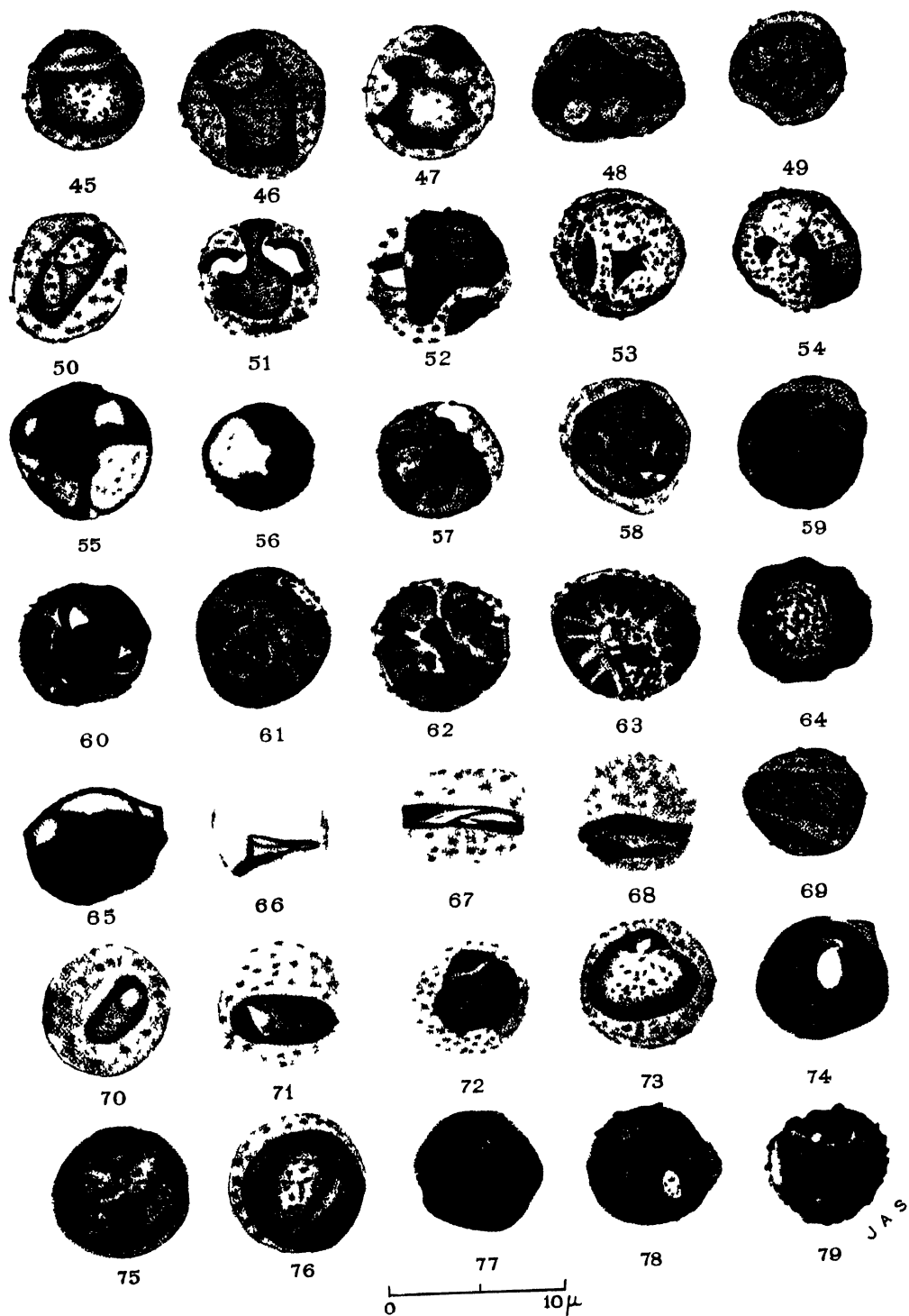
„ 72 and 73. Immature macro-gametocytes.

„ 74 to 76. Mature macro-gametocytes.

„ 77 to 79. Mature micro-gametocytes.

*In the reproduction of these plates the pigment granules have been made much too coarse, and cannot, therefore, be taken as an accurate guide to the appearances seen in stained specimens.

PLATE IV.



EFFECT OF 'SALINE AND FREE' AMMONIA ON THE
OVIPOSITION OF *ANOPHELES CULICIFACIES*
AND *ANOPHELES SUBPICTUS* (ROSSI).*

BY

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(From the Laboratory of the Malaria Survey of India, Karnal.)

[5th November, 1934.]

INTRODUCTION.

RECENT years have seen considerable developments in the physico-chemical aspects of hydro-biology. It has been established that the distribution of aquatic fauna largely depends on the chemical properties of water.

In the case of some anophelines it has been noted by a number of workers that, besides other factors, even very small amounts of free ammonia have marked inhibitory effects on their breeding. In this connection, Senior-White (1928) has shown that, except in the case of *rossi*-group, saline ammonia is inhibitory to anopheline breeding in amounts exceeding one part per million.

The exact mechanism which brings about this detrimental effect is not known. Senior-White (1928) proved that, whatever the inhibitory effect may be, it does not operate by hindering the hatching of eggs. Thus it appears that the female anophelines either do not oviposit on strongly ammoniacal waters, or the young larvæ fail to survive when placed in similar unfavourable situations.

While discussing the results of her experiments on *Anopheles tarsimaculatus*, Beattie (1932) has concluded that, if a pool is contaminated by urine, the female mosquito may avoid it because of an ammoniacal smell which may be perceptible to her. Beattie (1932) further contends that, from the results she has obtained in Trinidad, the ammonia-nitrogen is considered to have some effect upon the oviposition of *Anopheles tarsimaculatus*, but no effect on larval growth. This has also been previously shown by other workers (Beklemishev and Mitrofanova, 1926). They note that anopheline eggs are seldom, if ever, found in nature, in waters in which the larvæ cannot mature, whereas in similar

* Research work carried out under a grant from the International Health Division of the Rockefeller Foundation, New York.

water under the laboratory conditions eggs can hatch and the larvæ develop up to a certain extent. This fact, they assert, indicates that under natural conditions the distribution of larvæ is governed by the discrimination of the ovipositing adults.

There is no systematic record of any experimental work to indicate whether the female mosquitoes do show a selective action during oviposition and therefore do not lay eggs on highly ammoniacal waters. In the present studies are embodied the results of experiments to determine the extent to which the females of *Anopheles culicifacies* and *Anopheles subpictus* can use discrimination during oviposition on waters of varying ammonia contents.

I take this opportunity to thank Lieut.-Colonel J. A. Sinton, I.M.S., Director, Malaria Survey of India, for the great interest he has shown during the progress of this research work. He has also very kindly lent me several of the very important papers which had special bearing on this problem. This work was conducted at the Ross Field Experimental Station for Malaria at Karnal, with a Fellowship grant from the Rockefeller Foundation, New York City (U. S. A.), in the International Health Division, which is gratefully acknowledged. I also wish to express my thanks to the Indian Research Fund Association for the facilities for work which I have received through the Malaria Survey of India. In the end, I wish to sincerely thank my friend, Dr. B. N. Ghosh, D.Sc., Chemist, Malaria Survey of India, for the very valuable help I have received from him in devising methods to analyse the samples of water.

MATERIAL AND TECHNIQUE.

The gravid females of *Anopheles culicifacies* and *Anopheles subpictus* were collected from the Imperial Cattle Breeding and Dairy Farm at Karnal. These were confined in cages made of mosquito-netting and of the dimensions 4 feet \times 3½ feet and 1½ feet \times 1½ feet. Petri dishes of a standard size (1 inch \times 6½ inches) containing solutions, of varying strengths, of ammonium sulphate and ammonium carbonate in distilled water, were placed inside the cages. The gravid females were thus allowed the opportunity to oviposit on solutions of ammonium salts containing varying amounts of free ammonia. The experiments were kept over-night and the numbers of eggs laid by the female mosquitoes were recorded for each dish. In some of the experiments samples of water of varying ammonia contents were collected from outside and used in the Petri dishes. Thus it was possible to compare the results obtained in each case when solutions of ammonium salts and when samples of water from natural breeding places were employed.

A general survey of the breeding places of the two anopheline species (*Anopheles culicifacies* and *A. subpictus*), spread over a period of two months, was also carried out, and a record was kept of the amounts of 'saline and free' ammonia present in those waters.

DETERMINATION OF 'SALINE AND FREE' AMMONIA IN A SAMPLE OF WATER.

For the precise estimation of ammonia the more elaborate method of Wanklyn is usually adopted. But as the author was working single-handed and a very large number of samples had to be analysed, a much simpler

method was devised by Dr. B. N. Ghosh, D.Sc., Chemist, Malaria Survey of India. The method is described below :—

(1) 'Make a solution of 2 gms. of ammonium sulphate in 500 c.c. of water.

(2) Take 1 c.c. of this solution and dilute it to 200 c.c. and call it (A)—the stock solution.

(3) To make the different standards for comparison, take 1, 2, 3 or more c.c. of solution (A) in a Nessler cylinder (50 c.c. capacity), and add to this 2 c.c. of Nessler's reagent*. Dilute each separate amount up to 20 c.c. by the addition of distilled water.

(4) Now take 18 c.c. of the water to be examined in another Nessler cylinder of the same capacity, add to it 2 c.c. of Nessler's reagent and compare the colour with the solutions prepared from (A) plus Nessler's reagent diluted to 20 c.c. with distilled water. The distilled water used must be quite free from ammonia'.

There is, however, one flaw in this method. As the technique involves, and depends only on, the comparison of the colours of the water samples examined, it is only practicable when the clear samples of water are analysed. Bearing this in mind, only clear and transparent samples of water were considered, while all the turbid and muddy samples had to be discarded.

CALCULATIONS.

One c.c. of ammonium sulphate (strong) solution contains $\frac{0.42}{500}$ gm. of ammonia-nitrogen or 0.00084 gm.

One c.c. of (A) solution contains $\frac{0.00084}{200}$ gm. of N_2 , or 0.0000042 gm. of N_2 .

Suppose we need 'X' c.c. of solution (A) to match the colour of the water sample.

Then $\frac{'X'}{20} = \frac{18}{20}$, or 1 c.c. of water sample contains $\frac{'X'}{18}$ into 0.0000042 gm. of ammonia-nitrogen which is equal to 'Y'. By multiplying 'Y' with 1,000,000 will give us the amount of ammonia-nitrogen present in parts per million.

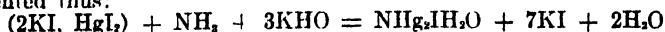
Now from the amount of ammonia-nitrogen present in a sample of water we can easily deduce the amount of 'saline and free' ammonia present in parts per million.

EXPERIMENTAL WORK.

(1) OVIPOSITION EXPERIMENTS ON *ANOPHELES CULICIFACIES*.

The gravid females of *Anopheles culicifacies* were confined in a large cage of the size 4 feet \times 3½ feet. In captivity this species of mosquito readily

* The Nessler's reagent consists of a saturated solution of periodide of mercury in distilled ammonia-free water, the whole being rendered alkaline with caustic potash. When this reagent is applied to a solution containing ammonia, it imparts a colour varying from faint yellow to a reddish brown, or even causes a precipitate, according to the amount of ammonia present. This reaction is due to the formation of ammonio-mercuric iodide and is represented thus:



The solution of Nessler's reagent should have a very faint yellow colour. If, however, it is colourless and 'non-sensitive' this can be remedied by adding a drop or two of a saturated solution of corrosive sublimate

oviposits and there has been no difficulty whatsoever in getting them to lay eggs in the laboratory. In the first series of these experiments water samples from the ponds were brought in glass jars with tightly fitting glass stoppers. Free ammonia present in the water was estimated and the water was then transferred to the Petri dishes in the cage. The results of the experiments have been tabulated below :—

TABLE I.

Number of Petri dish.	Number of females kept.	Free ammonia, p.p.m.	Number of eggs laid.	REMARKS.
1	18	28	348	
2	18	05	320	
3	18	Tap water	397	

The females appeared to have oviposited indiscriminately on the water samples and almost the same number of eggs was laid over-night in each case. Three more samples of water with varying free ammonia contents were obtained, and the gravid females were allowed to oviposit in the cage. The following table gives the results of the experiments :—

TABLE II.

Number of Petri dish.	Number of females kept.	Free ammonia, p.p.m.	REMARKS.
1	15	84	Eggs not laid.
2	15	07	Eggs laid.
3	15	04	" "

In the dish no. 1, the females failed to lay eggs, possibly because of the presence of free ammonia (8.4 p.p.m.) in great excess. In the other two cases, however, the eggs were laid freely.

The above experiments, however, were not considered enough from which to draw any definite conclusions. Consequently another series of experiments was arranged. In this case aqueous solutions of ammonium carbonate of various strengths were substituted in the dishes and the following results were obtained :—

TABLE III.

Series (1).

Date of experiment.	Number of Petri dish.	Number of females kept.	Free ammonia, p.p.m.	Number of eggs laid.
13-6-1933	1	21	1.5	229
"	2	21	0.45	250
"	3	21	0.16	322
"	4	21	Aq. dist.	295

TABLE III—concl'd.
Series (2).

Date of experiment.	Number of Petri dish.	Number of females kept.	Free ammonia, p.p.m.	Number of eggs laid.
14-6-1933	1	17	25	497
"	2	17	15	169
"	3	17	0.45	378
"	4	17	0.16	128

Series (3).

17-6-1933	1	27	5.0	366
"	2	27	25	154
"	3	27	0.45	No eggs laid.
"	4	27	Tap water	175

Series (4).

18-7-1933	1	9	13.2	No eggs laid
"	2	9	3.1	146
"	3	9	Tap water	139

Series (5).

22-7-1933	1	62	6.6	102
"	2	62	3.1	492
"	3	62	2.2	192
"	4	62	0.13	120
"	5	62	0.13	132
"	6	62	Tap water	215

(II) OVIPOSITION EXPERIMENTS ON *ANOPHELES SUBPICTUS*.

Anopheles subpictus (rossi) is well known to be capable of breeding in nature in comparatively foul water. It was therefore thought interesting to perform some more experiments, and observe if the gravid females of this species would lay eggs on highly ammoniacal waters. If so, what would be the limit up to which they would do so under laboratory conditions.

The following table will show the details of experiments on *Anopheles subpictus* performed under conditions very similar to those used for *Anopheles culicifacies* :—

TABLE IV.
Series (1).

Date of experiment.	Number of Petri dish.	Number of females kept.	Free ammonia, p.p.m.	Number of eggs laid.	REMARKS.
12-6-1933	1	34	0.45	1,332	
"	2	34	0.16	552	
"	3	34	Aq. dist.	180	

TABLE IV—concl'd.

Series (2):

Date of experiment.	Number of Petri dish.	Number of females kept.	Free ammonia, p.p.m.	Number of eggs laid.	REMARKS.
15-6-1933	1	14	8.8	548	
"	2	14	5.0	353	
"	3	14	2.5	516	
"	4	14	0.45	362	

Series (3).

28-6-1933	1	13	8.8	242	
"	2	13	5.0	58	
"	3	13	2.5	453	
"	4	13	0.72	..	Eggs not laid
"	5	13	0.36	..	" " "
"	6	13	Tap water	..	" " "
"	7	13	Aq dist	..	" " "

Series (4).

29-6-1933	1	22	20.16	..	Eggs not laid
"	2	22	8.8	Not recorded	Eggs laid.
"	3	22	5.0	" "	" "
"	4	22	2.5	" "	" "
"	5	22	1.2	" "	" "
"	6	22	1.0	..	Eggs not laid
"	7	22	0.3	..	" " "
"	8	22	Aq dist.	..	" " "

It is clear from the above experiments that the females of *Anopheles subpictus* (rossi) will oviposit in the laboratory on water with 'free' ammonia contents up to 8.8 p.p.m. Above this strength the mosquitoes failed to lay eggs in the laboratory. Perhaps in nature, where there is much wider selection and such high values of free ammonia are rare, the females of this species are not likely to exert any profound choice. Thus we may find only solitary instances of the presence of larvæ when the ammonia contents in water range about 8 p.p.m.

When a limited and a small range of low ammonia content was given, the mosquitoes laid eggs in all cases. As for example in Series (1), Table IV, the females laid eggs in all the three cases when the range of free ammonia did not exceed 0.45 p.p.m. On raising this limit to 8.8 p.p.m., and when the females had the free choice to lay eggs in dishes with free ammonia 5.0, 2.5, 0.72, 0.36 p.p.m., tap and distilled water respectively, it was found that they readily oviposited on the dishes with higher range of saline ammonia [Series (3) and (4), Table IV]. Curiously enough, it was also observed that the mosquitoes did not lay any eggs on water with free ammonia less than 1 p.p.m., when given a wide choice [*vide* Table IV, Series (3) and (4)]. This appears to indicate that *Anopheles subpictus* prefers to oviposit on places

where the water is comparatively foul. At least the presence of free ammonia up to 8·8 p.p.m. is in no way inhibitory to oviposition by this insect.

THE DISTRIBUTION OF THE LARVÆ OF *ANOPHELES CULICIFACIES* AND *A. SUBPICTUS* IN NATURAL POND WATER.

To ascertain the distribution of the larvæ of *Anopheles culicifacies* and *A. subpictus* in natural pond water, a survey was conducted and analyses carried out of the pond water collected from different places in relation to anopheline breeding.

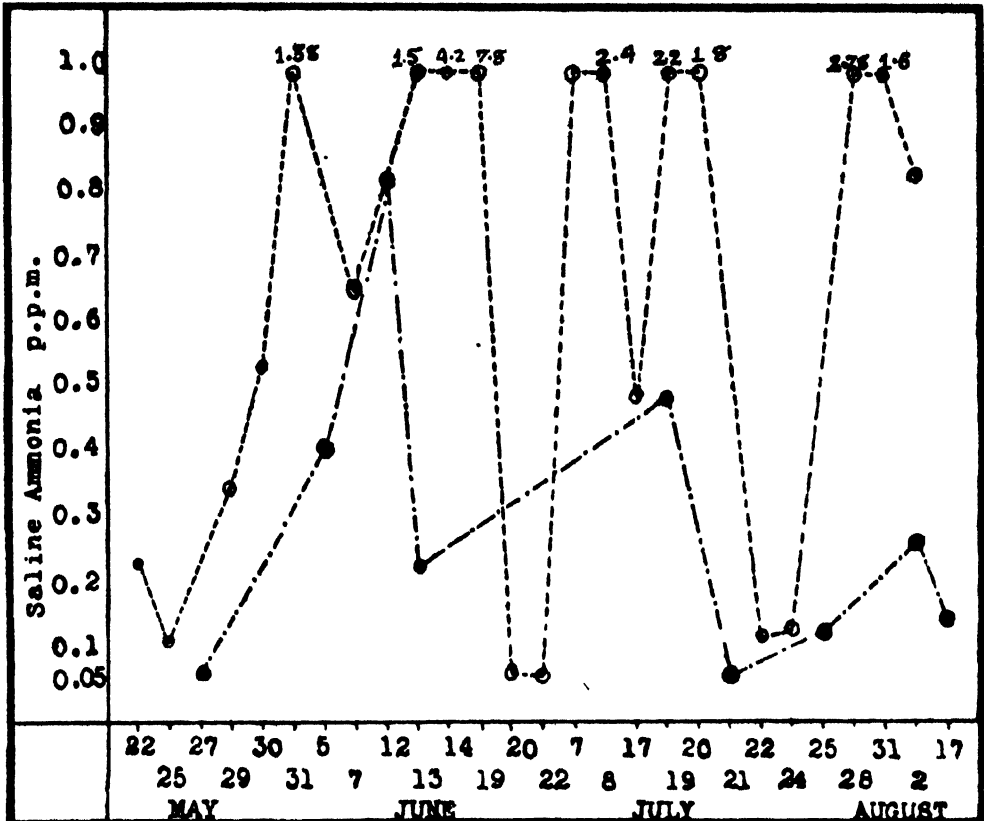
The results of this investigation have been shown in the following table and the accompanying graph :—

TABLE V.

Date of collection.	Larval species found.	Saline and free ammonia, p.p.m.
22-5-1933	<i>subpictus</i>	0·24
25-5-1933	"	0·12
27-5-1933	<i>culicifacies</i>	0·072
29-5-1933	<i>subpictus</i>	0·36
30-5-1933	"	0·55
31-5-1933	"	1·38
5-6-1933	<i>culicifacies</i>	0·42
7-6-1933	<i>subpictus</i>	0·66
12-6-1933	<i>culicifacies</i>	0·84
13-6-1933	<i>subpictus</i>	1·5
14-6-1933	"	4·2
19-6-1933	"	7·8
20-6-1933	"	7·8
22-6-1933	"	0·072
7-7-1933	"	0·07
8-7-1933	"	2·4
17-7-1933	"	0·5
19-7-1933	<i>culicifacies</i>	0·5
19-7-1933	<i>subpictus</i>	2·2
20-7-1933	"	1·8
21-7-1933	<i>culicifacies</i>	0·07
22-7-1933	<i>subpictus</i>	0·13
24-7-1933	"	0·14
25-7-1933	<i>culicifacies</i>	0·14
28-7-1933	<i>subpictus</i>	2·78
31-7-1933	"	1·60
2-8-1933	"	0·84
2-8-1933	<i>culicifacies</i>	0·28
17-8-1933	"	0·16

The results obtained from this survey have been most interesting. Whereas *Anopheles culicifacies* has not been found to breed in water which contains free ammonia more than one part per million, the other species, *A. subpictus*, was most commonly found when the free ammonia ranged even as high as two parts per million. In a few isolated instances, larvæ of this species have been recorded in very foul water.

Saline ammonia and distribution of *Anopheles culicifacies* and *Anopheles subpictus*.



- - - *A. subpictus*.
 . . . *A. culicifacies*

DISCUSSION.

It is a common belief that the selective instinct of insects during oviposition depends on sight, scent and taste. It is, however, extremely difficult to demonstrate experimentally which of these factors plays the chief rôle during this act. In the case of mosquitoes, it has been suggested that the females are guided by the sense of smell and also they are attracted by the presence of future food for the larvæ. Several other factors have also been mentioned in this connection.

It has been shown above that *Anopheles culicifacies* in nature has not been found breeding in water with saline ammonia contents more than one part per million (Table V). These results confirm the findings of Senior-White (1928), who has already shown that, except in the case of *rossi*-group, saline ammonia appears to be inhibitory to anopheline breeding in amounts exceeding one part per million. The larvæ of *Anopheles subpictus* are frequently met with in pond water with very high percentage of free ammonia.

During the course of oviposition experiments on *Anopheles culicifacies* in laboratory, it has been shown above that the females seem to oviposit indiscriminately within certain limits. It appears that this mosquito will lay eggs on water with saline ammonia ranging up to 6.6 parts per million. Above this limit, however, the females did not oviposit. But it has been shown above as a result of field work that the larvæ of *Anopheles culicifacies* are not found in nature in water with free ammonia exceeding one part per million. Senior-White (1928) has also shown experimentally that whatever the inhibitory effect may be of saline ammonia, it does not operate by hindering the hatching of eggs. It is therefore possible that the larvæ of *Anopheles culicifacies* may not survive for a long time in water with high percentage of saline ammonia. This aspect of the problem requires further elucidation.

The results obtained from experiments on *Anopheles subpictus* have been very interesting. It has been shown above that, up to a range of 8.8 parts per million, free ammonia is in no way inhibitory to oviposition in this insect. In nature, too, the larvæ have been found breeding up to very high ranges of free ammonia. It appears that the females have a marked tendency to oviposit on water with comparatively high range of saline ammonia above 2.5 parts per million under laboratory conditions. Whether this really occurs in nature it is very difficult to say, but it appears certain that saline and free ammonia has no ill effects on the larvæ of this species.

CONCLUSIONS AND SUMMARY.

Anopheles culicifacies oviposits indiscriminately in the laboratory within a certain range of ammonia. Above 6.6 p.p.m. of free ammonia in water the female mosquitoes refuse to oviposit if given a choice of other waters. In nature the larvæ have not been found in water with free ammonia exceeding one part per million. It appears, therefore, that either the females, in nature, do not lay any eggs on highly ammoniacal waters, or the young larvæ do not live long under such unfavourable conditions.

Anopheles subpictus will deposit eggs even when 8.8 parts per million of saline ammonia are present in water, and at the same time less ammoniacal water is available. Above this limit the females do not lay eggs. Our laboratory experiments seem to indicate that the females have a marked tendency to lay eggs on foul water.

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